

Antimicrobial Activity of Curcuminoids from *Curcuma longa* L. on Pathogenic Bacteria of Shrimp and Chicken

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ABSTRACT

Ethanol turmeric extract, hexane turmeric extract and curcuminoids (from the ethyl acetate extract of curcuminoids from *Curcuma longa*, which contained 86.5% curcumin) were evaluated for their inhibitory effect on 24 strains of pathogenic bacteria isolated from shrimp and chicken. The ethanol turmeric extract and the hexane turmeric extract showed inhibitory effects against 13 bacteria studied, namely: *Vibrio harveyi*, *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Staph. epidermidis*, *Staph. intermidis*, *Bacillus subtilis*, *B. cereus* and *Edwardsiella tarda*. The curcuminoids showed an inhibitory effect against eight bacteria, namely: *A. hydrophila*, *Str. agalactiae*, *Staph. aureus*, *Staph. epidermidis*, *Staph. intermidis*, *B. subtilis*, *B. cereus* and *Ed. tarda*. The minimum inhibitory concentrations of the ethanol turmeric extract, curcuminoids and hexane turmeric extract ranged from 3.91 to 125, 3.91 to 500 and 125 to 1000 ppt, respectively. The results appeared to indicate that the ethanol turmeric extract has high potential to inhibit some pathogenic bacteria of shrimp and chicken to a greater degree than curcuminoids and hexane turmeric extract.

Keywords: *Curcuma longa*, turmeric extract, curcuminoids, antibacterial activity, minimum inhibitory concentrations

INTRODUCTION

Plant extracts are used as local medicines in many parts of the world for combating various infectious diseases. Due to the increased resistance of many microorganisms towards the currently available commercial antibiotics, investigation of the chemical compounds in medicinal plants has become desirable (Yasunaka *et al.*, 2005). Turmeric (*Curcuma longa* Linn.), a plant of the family Zingiberaceae, grown mainly in Thailand, has been used in Thai herbal medicine for the

treatment of various skin diseases. There are several reports indicating a variety of pharmacological uses of turmeric, including: as an antioxidant (Masuda *et al.*, 2001, 2002; Das and Das, 2002), anti-protozoal activity (Araujo *et al.*, 1998), anti-microbial activity (Negi *et al.*, 1999), anti-venom activity (Ferreira *et al.*, 1992), anti-HIV activity (Sui *et al.*, 1993), anti-tumor activity (Ozaki *et al.*, 2000; Kim *et al.*, 2001), as an anti-inflammatory (Ammon and Wahl, 1991; Surh *et al.*, 2001), having a hepatoprotective effect (EL-Ansary *et al.*, 2006), as an anti-allergy (Yano

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et al., 2000), insect-repellant activity (Saju *et al.*, 1999), anti-ulcer activity (Rafatullah *et al.*, 1990), as an anti-dyspeptic (Deitelhoff *et al.*, 2002) and as an anti-depressant (Yu *et al.*, 2002). Thus, in this study, the efficiency of turmeric extracts, such as ethanol, hexane and curcuminoids, were evaluated for their inhibitory effect on 24 strains of pathogenic bacteria isolated from shrimp and chicken in an *in vitro* study and to determine the minimum inhibitory concentration (MIC) of these turmeric extracts and curcuminoids.

MATERIALS AND METHODS

Plant materials

Rhizomes of turmeric (*Curcuma longa* Linn.) were collected from Kanchanaburi province, Thailand. A voucher specimen (BK 63868) was deposited at the Bangkok Herbarium, Department of Agriculture, Bangkok, Thailand.

Extraction of active compounds from *Curcuma longa* Linn.

All curcuminoid analyses were carried out by the Natural Products and Organic Syntheses Research Unit (NPOS), Department of Chemistry, Faculty of Science, Kasetsart University, Thailand.

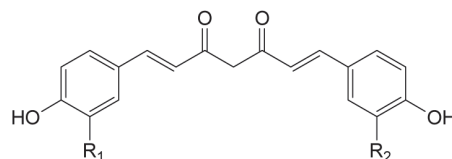
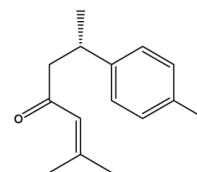
Ethanol turmeric extract

Dry rhizomes of *Curcuma longa* (1.15 kg) were extracted with ethanol (6 L) for 8 h using a soxhlet extractor. The solution was evaporated to dryness under vacuum to give ethanol turmeric extract. The ethanol turmeric extract was a dark red gum that weighed 230 g (20% dry weight).

Hexane turmeric extract and Isolation of curcuminoids

Dry rhizomes of *Curcuma longa* (1.0 kg) were extracted with hexane (6 L) for 10 h using a soxhlet extractor to give hexane turmeric extract as a yellow oil weighing 53.75 g (5.38% dry weight). The hexane solution was evaporated to

dryness by a rotary evaporator to give hexane turmeric extract containing 59.92% ar-turmerone as the major compound. The hexane turmeric extract was analyzed by GC-MS (Krittika *et al.*, 2007). The residue was then extracted with ethyl acetate (6 L) for 40 h using a soxhlet extractor. The ethyl acetate solution was concentrated, filtered and recrystallized with ethanol to produce 32.76 g and 32.28% curcuminoids as orange crystals, with a melting point of 176-178°C (Jayaprakasha *et al.*, 2005). The crystals contained 86.5% curcumin, 13.4% demethoxycurcumin and 0.1% bisdemethoxycurcumin by HPLC analysis. The curcuminoids were analyzed by HPLC (Marsin *et al.*, 1993).



Curcumin : $R_1 = R_2 = \text{OMe}$

Demethoxycurcumin : $R_1 = \text{H}, R_2 = \text{OMe}$

Bisdemethoxycurcumin : $R_1 = R_2 = \text{H}$

Antimicrobial inhibitory effects test

Bacterial strain and inoculum preparation

Twenty-four strains of pathogenic bacteria were used in the study. *Vibrio harveyi*, *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *Aeromonas hydrophila*, were isolated from black tiger shrimp (Lawhavit *et al.*, 2006). *Bacillus subtilis*, *B. cereus*, *Salmonella* serv. Typhi, *Salmonella* serv. Typhimurium, *Salmonella* serv. Enteritidis, were isolated from broilers and identified with DMST standard

antisera (Lawhavit *et al.*, 2007). *Streptococcus agalactiae* (DMST17129), *Staphylococcus aureus* (ATCC25923), *Staph. epidermidis* (ATCC 12228), *Staph. intermidis* (DMST 11465), *Edwardsiella tarda* (DMST 10596), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (DMST 557), were provided by the Faculty of Veterinary Technology, Kasetsart University, Thailand. *Proteus mirabilis*, *Shigella sonnei*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Erwinia carotovora* and *Citrobacter freundii* were supplied by the Department of Microbiology, Faculty of Science, Kasetsart University, Thailand. Each strain of bacteria was cultured on nutrient agar (Difco) followed by incubation at 37°C for 24 h. Each inoculum was adjusted in 0.85% NaCl to approximate McFarland standard No. 0.5 (approx. cell density 1.5×10^8 CFU/ml). Each bacterial inoculum was inoculated on Mueller Hinton Agar (MHA; Difco) using a swab plate technique.

Sensitivity testing

The Kirby-Bauer method (Bauer *et al.*, 1966) was used for sensitivity testing of the ethanol turmeric extract, hexane turmeric extract and curcuminoids. Turmeric extract samples were dissolved with dimethylsulfoxide (DMSO) at 1g per 1mL. DMSO was purchased from Crown Zellerbach, Camas, Wash. However, the minimal microbiocidal concentration of DMSO generally was several times higher than the minimal inhibitory concentration, except for certain species that appeared to be ultrasensitive to this agent, such as *Corynebacterium* sp., *Haemophilus influenzae*, *Pasteurella multocida*, *Herellea* sp., *Mycobacterium tuberculosis* Var. BCG, and *Microsporium audouini* (Basch and Gadebusch, 1968). Therefore, the solvent, pure DMSO, of each extract was used as a negative control and 30 µg per disc of tetracycline (Oxoid) was used as a positive control. Solutions were applied to sterile filter paper discs (Whatman grade AA discs size 6 mm in diameter) and placed on the surface of the assay plates, and then were incubated at 37°C for

24 h. Antibacterial activity was determined by the presence of an inhibition zone around the disc.

Determination of minimum inhibitory concentration (MIC)

The MIC for each sample was determined using the disc diffusion method. Concentrated extracts of turmeric (ethanol turmeric extract, hexane turmeric extract and curcuminoids) were added at two-fold serial dilution with DMSO (0.244 to 1000 ppt). Each diluted solution was applied to sterile filter paper discs (Whatman grade AA discs size 6 mm in diameter) and placed on the surface of the assay plates, which were then incubated at 37°C for 24 h. MIC values were taken as the lowest concentration of extract that completely inhibited bacterial growth or produced a clear zone on the disc that was bigger than the negative control (pure DMSO) disc after 24 h of incubation at 37°C.

RESULTS AND DISCUSSION

Sensitivity testing

Ethanol turmeric extract and hexane turmeric extract showed inhibitory effects for *V. harveyi*, *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *A. hydrophila*, *Str. agalactiae*, *Staph. aureus*, *Staph. epidermidis*, *Staph. intermidis*, *B. subtilis*, *B. cereus* and *Ed. Tarda*, but did not inhibit *Salmonella* serv. Typhi, *Salmonella* serv. Typhimurium, *Salmonella* serv. Enteritidis, *E. coli*, *P. mirabilis*, *P. vulgaris*, *Shi. sonnei*, *Ent. aerogenes*, *Kleb. pneumoniae*, *Er. carotovora* and *Cit. freundii* (Table 1). Negi *et al.* (1999) reported the antibacterial activity of turmeric oil extracted from spent turmeric oleoresin, which was separated into three fractions using column chromatography. These fractions showed antibacterial activity against *B. cereus*, *B. coagulans*, *B. subtilis*, *Staph. aureus*, *E. coli*, and *Pseudomonas aeruginosa*. Therefore, the results of the present study were similar to Negi *et al.* (1999), because the ethanol and hexane turmeric

extracts showed inhibitory effects for *B. cereus*, *B. subtilis*, *Staph. aureus*, and *E. coli*.

However, the curcuminoids showed inhibitory effects for *A. hydrophila*, *Str. agalactiae*, *Staph. aureus*, *Staph. epidermidis*, *Staph. intermidis*, *B. subtilis*, *B. cereus* and *Ed. Tarda*, but did not inhibit *V. harveyi*, *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *Salmonella serv. Typhi*, *Salmonella serv. Typhimurium*, *Salmonella serv. Enteritidis*, *E. coli*, *P. mirabilis*, *P. vulgaris*, *Shi. sonnei*, *Ent. aerogenes*, *Kleb. pneumoniae*, *Er. carotovora* and

Cit. freundii (Table 1).

Minimum inhibitory concentration of ethanol turmeric, hexane turmeric extract and orange crystals

The MIC value of turmeric extract was also investigated using disc diffusion methods. The MIC value of ethanol turmeric extract with *B. cereus*, *Str. agalactiae*, *V. harveyi*, *V. cholerae* (Figure 1), *V. alginolyticus*, *Staph. intermidis*, *B. subtilis*, *Staph. aureus*, *Staph. epidermidis*, *V. parahaemolyticus*, *A. hydrophila*, *V. vulnificus* and

Table 1 Results of screening for inhibitory effects, diameter of inhibition zone (mm) of ethanol turmeric extract, hexane turmeric extract and curcuminoids against 24 strains of pathogenic bacteria.

Pathogenic bacteria	Sample		
	Ethanol turmeric extract	Hexane turmeric extract	Curcuminoids
<i>Vibrio harveyi</i>	23.33 ± 0.09	15.72 ± 0.16	0
<i>Vibrio cholerae</i>	21.37 ± 0.49	14.28 ± 0.15	0
<i>Vibrio alginolyticus</i>	22.37 ± 0.26	14.73 ± 0.15	0
<i>Vibrio parahaemolyticus</i>	21.50 ± 0.29	14.72 ± 0.79	0
<i>Vibrio vulnificus</i>	22.23 ± 0.12	15.00 ± 0.26	0
<i>Aeromonas hydrophila</i>	17.45 ± 0.14	16.32 ± 0.45	14.12 ± 0.12
<i>Bacillus subtilis</i>	10.33 ± 0.49	8.74 ± 0.11	7.45 ± 0.23
<i>Bacillus cereus</i>	16.37 ± 0.49	8.65 ± 0.14	7.12 ± 0.29
<i>Salmonella serv. Typhi</i>	0	0	0
<i>Salmonella serv. Typhimurium</i>	0	0	0
<i>Salmonella serv. Enteritidis</i>	0	0	0
<i>Streptococcus agalactiae</i>	22.00 ± 0.18	21.11 ± 0.13	17.24 ± 0.24
<i>Staphylococcus aureus</i>	16.55 ± 0.42	9.87 ± 0.11	11.12 ± 0.23
<i>Staphylococcus epidermidis</i>	10.45 ± 0.10	7.00 ± 0.13	9.00 ± 0.17
<i>Staphylococcus intermidis</i>	19.57 ± 0.24	8.15 ± 0.26	9.45 ± 0.15
<i>Edwardsiella tarda</i>	11.00 ± 0.14	8.45 ± 0.23	7.12 ± 0.13
<i>Escherichia coli</i>	0	0	0
<i>Proteus vulgaris</i>	0	0	0
<i>Proteus mirabilis</i>	0	0	0
<i>Shigella sonnei</i>	0	0	0
<i>Enterobacter aerogenes</i>	0	0	0
<i>Klebsiella pneumoniae</i>	0	0	0
<i>Erwinia carotovora</i>	0	0	0
<i>Citrobacter freundii</i>	0	0	0

Note: 0 indicates there was no inhibition zone around the disc, which was the same as for the negative control.

Ed. tarda was 3.91, 7.81, 15.63, 15.63, 15.63, 15.63, 15.63, 31.25, 31.25, 31.25, 62.50, 125 and 125 ppt, respectively (Figure 2).

The MIC value of hexane turmeric extract with *Str. agalactiae*, *Staph. intermidis*, *B. subtilis*, *V. harveyi*, *V. alginolyticus*, *Staph. aureus*, *B. cereus*, *V. cholerae*, *A. hydrophila*, *Staph. epidermidis*, *Ed. tarda*, *V. parahaemolyticus* and *V. vulnificus* was 125, 125, 125, 250, 250, 250, 250, 500, 500, 500, 500, 1,000 and 1,000 ppt, respectively (Figure 3).

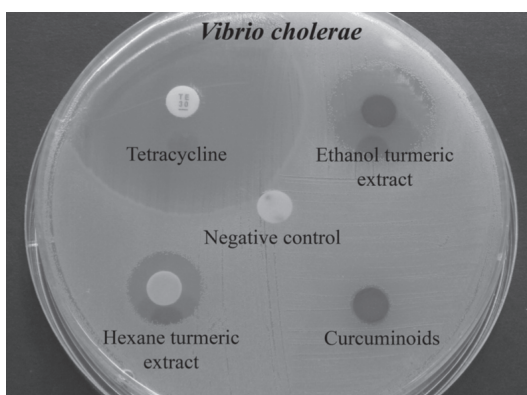


Figure 1 Inhibition zone of *Vibrio cholerae* with turmeric extracts, negative control and tetracycline.

The MIC value of curcuminoids with *Staph. aureus*, *B. cereus*, *Staph. epidermidis*, *Staph. intermidis*, *B. subtilis*, *A. hydrophila*, *Str. agalactiae* and *Ed. tarda* were 3.91, 15.63, 125, 125, 125, 250, 500 and 500 ppt, respectively (Figure 3), while all strains of *Vibrio* sp. tested were resistant to curcuminoids (Table 1). In the current study, curcuminoids produced better results with *Staph. aureus* than the experiment of Bhavanishankar and Srinivasamurthy (1979) in which curcumin (2.5-50 mg/mL) inhibited only *Staph. aureus*. However, *Salmonella* serv. Typhi, *Salmonella* serv. Typhimurium, *Salmonella* serv. Enteritidis, *E. coli*, *P. mirabilis*, *P. vulgaris*, *Shi. sonnei*, *Ent. aerogenes*, *Kleb. pneumonia*, *Er. carotovora* and *Cit. frundii* were resistant to ethanol turmeric extract, hexane turmeric extract and curcuminoids.

The results showed that the ethanol and hexane turmeric extracts gave better inhibitory effects on the pathogenic bacteria of shrimp than those of broilers. The results appear to indicate that the turmeric extract has high potential to inhibit some pathogenic bacteria. Bhavanishankar and Murthy (1986) and Lutomoski *et al.* (1974), showed that, several species of bacteria were

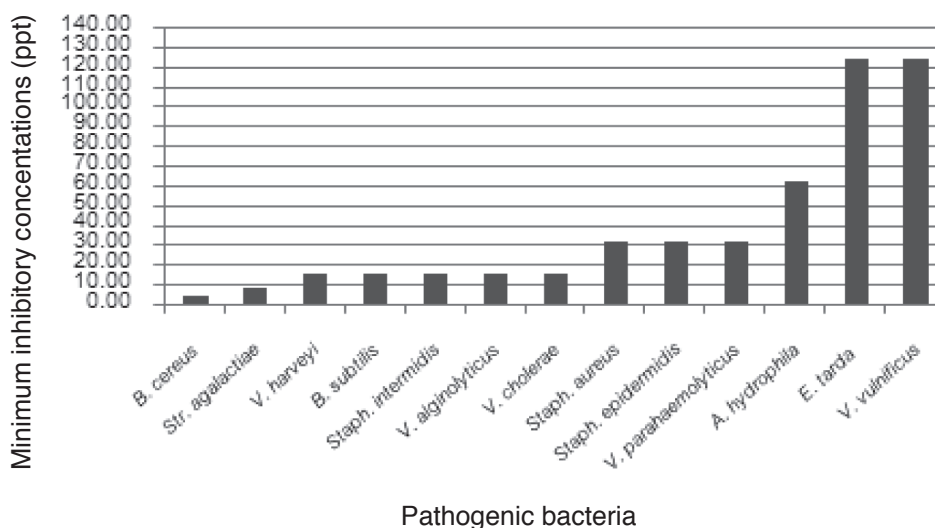


Figure 2 MIC values of ethanol turmeric extract against some pathogenic bacteria.

sensitive to turmeric extract. Therefore, this study provided additional information for more species of bacteria on the inhibitory effects of turmeric extract. A mixture of the turmeric extracts could be a potentially useful source of antimicrobial compounds.

CONCLUSIONS

Turmeric (*Curcuma longa* Linn.) has been used in Thai herbal medicine for the treatment of various skin diseases. There are several reports indicating a variety of pharmacological activities

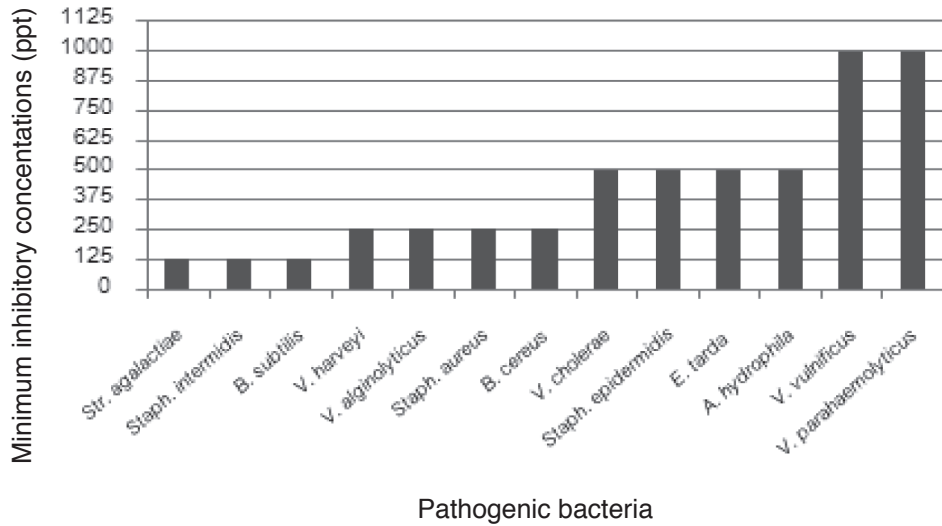


Figure 3 The MIC values of hexane turmeric extract against some pathogenic bacteria.

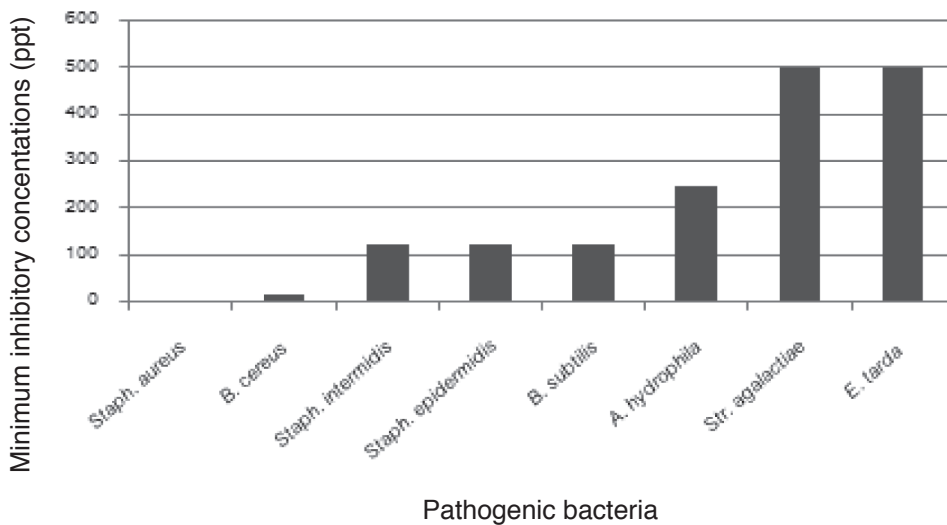


Figure 4 MIC values of curcuminoids for some pathogenic bacteria.

of turmeric. Thus, in this study, the efficiency of turmeric extracts, such as ethanol, hexane and curcuminoids, were evaluated for their inhibitory effect on 24 strains of pathogenic bacteria isolated from shrimp and chicken. The minimum inhibitory concentrations of the ethanol turmeric extract, curcuminoids and hexane turmeric extract ranged between 3.91 to 125, 3.91 to 500 and 125 to 1000 ppt, respectively. The ethanol turmeric extract had high potential to inhibit some pathogenic bacteria of shrimp and chicken to a greater degree than curcuminoids and hexane turmeric extract. This study provided more information on the inhibitory effect of ethanol turmeric extracts on pathogenic bacteria isolated from shrimp and chicken.

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