Inhibitory effects of Turmeric (Curcuma longa Linn.) extracts on some human and animal pathogenic bacteria

Pornchai Sincharoenpokai, Ong-ard Lawhavinit, Patcharee Sunthornandh, Ngampong Kongkathip, Suriyan Sutthiprabha, Boonsong Kongkathip

ABSTRACT

Inhibitory effects of the ethanol turmeric extract, hexane turmeric extract and curcuminoids from the ethyl acetate extract which contained curcumin 86.5 %, were examined on some human and animal pathogenic bacteria by the Kirby- Bauer method. The ethanol turmeric extract and the hexane turmeric extract showed inhibitory effects against the following 13 bacteria; V. harveyi, V. cholera, V. alginolyticus, V. parahaemolyticus, V. vulnificus, A. hydrophila, Str. agalactiae, Staph. aureus, Staph. epidermidis, Staph. intermidis, B. subtilis, B. cereus and Ed. tarda. The curcuminoids showed inhibitory effect against 8 bacteria which were A. hydrophila, Str. agalactiae, Staph. aureus, Staph. epidermidis, Staph. intermidis, B. subtilis, B. cereus and Ed. tarda. The MICs of the ethanol turmeric extract, hexane turmeric extract, and curcuminoids are expected to be useful for the treatment of pathogenic bacteria.
turmeric extract and curcuminoids ranged from 3.91-125, 125-1000 and 3.91-500 ppt, respectively. The results of this investigation appear to indicate that turmeric extract has high potential of inhibitory effect to some human and animal pathogenic bacteria.

Key Words: Curcuma longa Linn., turmeric extract, curcuminoids, antibacterial activity, MICs
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INTRODUCTION

Plant extracts are used as folk medicines in many parts of the world for combating various infectious diseases. Due to increase 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 Zingiberaceae family, grown mainly in Thailand, has been used in Thai traditional medicine for the treatment of various skin diseases. There are several reports indicating a variety of pharmacological activities of turmeric, such as antioxidant (Masuda et al., 2001, 2002, Das and Das, 2002), anti-protozoal (Araujo et al., 1998), anti-microbial (Negi et al., 1999), anti-venom (Ferreria et al., 1992), anti-HIV (Sui et al., 1993), anti-tumor (Ozaki et al., 2000, Kim et al., 2001), anti-inflammatory (Ammon and Wahl, 1991, Surh et al., 2001), hepatoprotective (EL-Ansary et al., 2006), anti-allergic (Yano et al., 2000), insect-repellant (Venugopal and Saju, 1999), anti-ulcer (Rafatullah et al., 1990), anti-dyspeptic (Deitelhofft et al., 2002) and anti-depressant (Yu et al., 2002). Thus, in this study, the potential of turmeric extracts were screened the potential of turmeric extracts to inhibit 24 pathogenic bacteria and investigated the minimum inhibitory concentrations (MICs) of these turmeric extracts and curcuminoids.

MATERIALS AND METHODS

Plant materials

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

Extraction of active compounds from Curcuma longa Linn.

1. Ethanol turmeric extract

The dry rhizomes of Curcuma longa (1.15 kg) were extracted with ethanol (6 L) for 8 h by soxhlet extractor. The solution was evaporated to dryness under vacuum to give ethanol turmeric extract.
2. Hexane turmeric extract and Isolation of curcuminoids

The dry rhizomes of *Curcuma longa* (1.0 kg) were extracted with hexane (6 L) for 10 h by soxhlet extractor. The hexane solution was evaporated to dryness by a rotary evaporator to give hexane turmeric extract. 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 by soxhlet extractor. The ethyl acetate solution was concentrated, filtered and recrystallized with ethanol to give curcuminoids as orange crystals. The curcuminoids was analyzed by HPLC (Marsin et al., 1993).

Antimicrobial inhibitory effects test

1. Bacterial strains and inoculum preparation

Twenty-four strains of human and animal pathogenic bacteria were used: *Vibrio harveyi*, *V. cholera*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Staph. epidermidis*, *Staph. intermidis*, *Bacillus subtilis*, *B. cereus*, *Edwardsiella tarda*, *Salmonella Typhi*, *S. Typhimurium*, *S. Enteritidis*, *Escherichia coli*, *Proteus mirabilis*, *P. vulgaris*, *Shigella sonnei*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Erwinia carotovora* and *Citrobacter frundii*. Each strain of bacteria was cultured on nutrient agar followed by incubation at 37°C for 24 h. The inoculum was adjusted in 0.85% NaCl approximately McFarland standard No. 0.5 (approx. cell density 1.5x10^8 CFU/ml). Bacterial inoculums were inoculated on Mueller Hinton Agar (MHA) using swab plate technique.

2. Sensitivity testing

The Kirby-Bauer method (Bauer et al., 1966) was used for sensitivity test of ethanol turmeric extract, hexane turmeric extract and curcuminoids. Turmeric extract was dissolved with DMSO. 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. The solvent of each extract was used as a negative control. Antibacterial activity was determined by inhibition zone around the disc.

3. Determination of minimum inhibitory concentrations (MICs)

The MICs 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 (0.244 to 1000 ppt). Each solution dilution 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. MICs values were taken as the lowest concentration of extract that completely inhibited bacterial growth after 24 h of incubation at 37 °C.
RESULTS AND DISCUSSIONS

Ethanol turmeric extract

The dry rhizomes (1.15 kg) of *Curcuma longa* from Kanchanaburi province were extracted with ethanol (6 L) for 8 h by soxhlet extractor to afford the ethanol turmeric extract (230 g, 20 % dry wt.) as a dark red gum.

Extraction of hexane turmeric extract and isolation of curcuminoids

The dry rhizomes (1.0 kg) of *Curcuma longa* from Kanchanaburi province were extracted with hexane (6 L) for 10 h by soxhlet extractor to give hexane turmeric extract (53.75 g, 5.38 % dry wt.) as yellow oil. By GC-MS analysis, the hexane turmeric extract contained ar-turmerone (59.92 %) as a major compound. The residue was then extracted with ethyl acetate (6 L) for 40 h by soxhlet extractor. The ethyl acetate solution was concentrated, filtered and recrystallized with ethanol to give curcuminoids (32.76 g, 3.28 % dry wt) as orange crystals, m.p. 176-178 °C (Jayaprakasha et al., 2005), which contained curcumin 86.5%, demethoxycurcumin 13.4%, and bisdemethoxycurcumin 0.1% by HPLC analysis.

![ar-turmerone](image)

**ar-turmerone**

![curcumin](image)

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

Sensitivity testing

From Table 1, Ethanol turmeric extract and hexane turmeric extract showed inhibitory effects for *V. harveyi*, *V. cholera*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *A. hydrophila*, *Str. agalactiae*, *Staph. aureus*, *Staph. epidermidis*, *Staph. intermidis*, *B. subtilis*, *B. cereus* and *Ed. tarda* but not inhibit *S. Typhi*, *S. Typhimurium*, *S Enteritidis*, *E. coli*, *P. mirabilis*, *P. vulgaris*, *Shi. sonnei*, *Ent. aerogenes*, *Kleb. pneumoniae*, *Er. carotovora* and *Cit. frundii*. The result was similar to that reported by Negi et al. (1999) who reported the antibacterial activity of turmeric oil. The said authors extracted the oil from the spent turmeric oleoresin and it was separated into three fractions using
column chromatography. These fractions were tested for antibacterial activity by pour plate method against *B. cereus*, *B. coagulans*, *B. subtilis*, *Staph. aureus*, *E. coli*, and *Pseudomonas aeruginosa*.

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 not inhibit *V. harveyi*, *V. cholera*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *S. Typhi*, *S. Typhimurium*, *S. Enteritidis*, *E. coli*, *P. mirabilis*, *P. vulgaris*, *Shi. sonnei*, *Ent. aerogenes*, *Kleb. pneumoniae*, *Er. carotovora* and *Cit. frundii* (Table 1).

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

The minimum inhibitory concentrations (MICs) using disc diffusion methods of turmeric extract were also investigated. The MICs values of ethanol turmeric extract against *B. cereus*, *Str. agalactiae*, *V. harveyi*, *V. cholera*, *V. alginolyticus*, *Staph. intermidis*, *B. subtilis*, *Staph. aureus*, *Staph. epidermidis*, *V. parahaemolyticus*, *A. hydrophila*, *V. vulnificus* and *Ed. tarda* was 3.91, 7.81, 15.63, 15.63, 15.63, 15.63, 31.25, 31.25, 31.25, 62.50, 125 and 125 ppt, respectively (Fig 1).

The MICs values of hexane turmeric extract against *Str. agalactiae*, *Staph. intermidis*, *B. subtilis*, *V. harveyi*, *V. alginolyticus*, *Staph. aureus*, *B. cereus*, *V. cholera*, *A. hydrophila*, *Staph. epidermidis*, *Ed. tarda*, *V. parahaemolyticus* and *V. vulnificus* was 125, 125, 125, 250, 250, 250, 250, 500, 500, 500, 1000 and 1000 ppt, respectively (Fig 2).

The MIC values of curcuminoids against *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, 500 and 500 ppt, respectively while all strains of *Vibrio* sp. tested were resistant to curcuminoids (Fig 3). In the present studies the result of curcuminoids on *Staph. aureus* was better than the experiment of Bhavanishankar and Srinivasamurthy (1979) in which curcumin (2.5–50 mg/ml) inhibited only *Staph. aureus*. 
<table>
<thead>
<tr>
<th>Pathogenic bacteria</th>
<th>Sample</th>
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<tbody>
<tr>
<td></td>
<td>Ethanol turmeric extract</td>
</tr>
<tr>
<td>Vibrio harveyi</td>
<td>+</td>
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<tr>
<td>Vibrio cholerae</td>
<td>+</td>
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<tr>
<td>Vibrio alginolyticus</td>
<td>+</td>
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<tr>
<td>Vibrio parahaemolyticus</td>
<td>+</td>
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<tr>
<td>Vibrio vulnificus</td>
<td>+</td>
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<tr>
<td>Aeromonas hydrophila</td>
<td>+</td>
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<tr>
<td>Streptococcus agalactiae</td>
<td>+</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
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<tr>
<td>Staphylococcus epidermidis</td>
<td>+</td>
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<tr>
<td>Staphylococcus intermidis</td>
<td>+</td>
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<tr>
<td>Bacillus subtilis</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>+</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>+</td>
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<tr>
<td>Salmonella Typhi</td>
<td>_</td>
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<tr>
<td>Salmonella Typhimurium</td>
<td>_</td>
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<tr>
<td>Salmonella Enteritidis</td>
<td>_</td>
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<tr>
<td>Escherichia coli</td>
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<tr>
<td>Proteus mirabilis</td>
<td>_</td>
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<tr>
<td>Proteus vulgaris</td>
<td>_</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>_</td>
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<tr>
<td>Enterobacter aerogenes</td>
<td>_</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>_</td>
</tr>
<tr>
<td>Erwinia carotovora</td>
<td>_</td>
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<tr>
<td>Citrobacter freundii</td>
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</tbody>
</table>

+ = have inhibition zone
_ = no inhibition zone

Table 1 The results of screening for inhibitory effects of ethanol turmeric extract, hexane turmeric extract and curcuminoids against 24 pathogenic bacteria.
Fig 1. The MICs values of ethanol turmeric extract against some pathogenic bacteria

Fig 2. The MICs values of hexane turmeric extract against some pathogenic bacteria

Fig 3. The MICs values of curcuminoids against some pathogenic bacteria
However, S. Typhi, S. Typhimurium, S. 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.

CONCLUSIONS

Comparing the activities of ethanol turmeric extract, hexane turmeric extract and curcuminoids it seems that the active compounds could belong to the hydrophilic group rather than to the lipophilic. The ethanol turmeric extract was the most inhibitory to the tested bacteria. The results of this investigation appear to indicate that the turmeric extract has high potential of inhibitory effects.

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LITERATURE CITED


