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# Research Article
# Potential Antibacterial Activity of Ethanolic *Curcuma longa* L. Rhizome Extract Against Antibiotic-Resistant Bacteria

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## Abstract

**Background and Objective:** *Curcuma longa* L. rhizomes are the source of many bioactive compounds such as antitumor, antidepressant, antibacterial, anti-aging and antidiabetic. Due to the growing problem of antibiotic-resistant bacteria, it is necessary to find new sources of antibiotics. This research aimed to investigate the antibacterial activity of ethanolic *Curcuma longa* L. rhizomes extract against *Proteus mirabilis, Acinetobacter baumannii* and Multidrug-Resistant *Klebsiella pneumoniae* (MDR-K). **Materials and Methods:** Dry *Curcuma longa* L. rhizomes were extracted with ethanol. The agar diffusion method was used as the primary screening of antibacterial activity determination. The broth dilution method was used to measure the MIC and MIC of the extract. **Results:** It presented the largest diameter of the inhibition zone at 0.9 mm against *Proteus mirabilis*, followed by 0.8 mm against MDR-K. The lowest MIC and MBC values were at 0.048 and 0.39 mg $mL^{-1}$ against *Proteus mirabilis*, followed by 0.195 and 6.25 mg $mL^{-1}$ against MDR-K. The ethanolic *Curcuma longa* L. rhizomes extract did not affect *Acinetobacter baumannii*. **Conclusion:** The new finding of this research was that the ethanolic extract from *Curcuma longa* L. rhizomes can eliminate *Proteus mirabilis* and MDR-K that can be applied to treating antibiotic-resistant bacterial infectious diseases in the hospital.

Key words: Antibacterial activity, *Curcuma longa* L. extract, multidrug-resistant *Klebsiella pneumoniae*, *Proteus mirabilis*, ethanolic extraction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Antibiotic-resistant bacterial infection is a major problem of global health. The list of twelve antibiotic-resistant bacteria included multi-drugs resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, cephalosporin-resistant *Neisseria gonorrhoeae*, vancomycin-resistant *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Enterobacteriaceae*, clarithromycin-resistant *Helicobacter pylori,* fluoroquinolone-resistant *Campylobacter* spp., fluoroquinolone-resistant *Salmonellae*, penicillin-non-susceptible *Streptococcus pneumoniae*, ampicillin-resistant *Haemophilus influenzae* and fluoroquinolone-resistant *Shigella* spp.[1]. They are many mechanisms by which these bacteria were generated to prevent antibiotic action such as target site modification[2], limiting uptake of an antibiotic, inactivation of an antibiotic and active efflux of an antibiotic[3]. New types of antibiotics are required.

Plants are sources of natural antibiotics that are currently being studied for new sources of antibiotics[4]. *Curcuma longa* L. or turmeric is a plant in the Zingiberaceae family which found in Asia, China, Taiwan, Japan, Burma, India, Indonesia and Thailand. In Thailand, turmeric is found growing in every region of the country. Turmeric is a medicine and a spice that can be added to curry spice food. Turmeric helps in deodorizing the fishy smell, is a seasoning herb and helps heal wounds as well. Turmeric is a rhizomes underground plant that has medicinal properties. The dried turmeric rhizome contained various biochemical content such as anthocyanins, phenols and tannins. Various biological activities of turmeric and its compound were reported such as antigrowth, anti-inflammatory, anti-arthritic, antioxidant, anti-atherosclerotic, antitumor, antidepressant, antibacterial, anti-aging, antidiabetic, antiprotozoal, would-healing, antifungal, antiviral activity, antidotal effects and antibiofilm[5-8]. Curcumin or diferuloylmethane is the main bioactive compound of turmeric as an antibacterial agent.

Many researchers reported antimicrobial activity from turmeric extract. Kim *et al.*[9], reported 0.125-2 mg $mL^{-1}$ ethyl acetate extract of *Curcuma longa* has antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA). Adamczak *et al.*[10] presented that curcumin was inhibiting the growth of *Streptococcus pyogenes*, methicillin-sensitive *S. aureus*, *Acinetobacter lwoffii*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Hussain and Atray[11] reported that ethanolic extract of *Curcuma longa* rhizome can be inhibited the growth of *Klebsiella pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. viridans* and *E. faecalis*. Not only turmeric extract was used to determine the antibacterial activity against antibiotic-resistant bacteria, but other plant extracts were also reported. *Cathormion umbellatum* extracts were reported that about the bactericidal activity against *A. baumannii*, *Stenotrophomonas maltophilia*, *E. faecalis*, *Burkholderia pseudomallei*, *P. mirabilis*, MDR-K, Colistin-resistant *P. aeruginosa*[12]. From the above information, only some reports about turmeric's antibacterial activity against antibiotic-resistant bacteria. This research aimed to evaluate the potential antibacterial activity of ethanolic *Curcuma longa* L. rhizome extract against antibiotic-resistant bacteria.

## MATERIALS AND METHODS

**Study area:** This research was performed from October, 2022 to February, 2023 in the Department of Biology, Faculty of Science and Technology, Rajabhat Maha Sarakham University, Maha Sarakham and the Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

***Curcuma longa* L. collection and preparation:** *Curcuma longa* were collected from the organic medical farm located in Maha Sarakham, Thailand. *Curcuma longa* were washed with tap water until clean and cut into small pieces before drying at 50°C for 3 days using a hot air oven (POL-EKO-APARATURA company, Wodzisław Śląski, Poland). The dried *Curcuma longa* sample was grounded into powder before extraction.

***Curcuma longa* L. extraction:** Fifty grams of *C. longa* powder were extracted with 100 mL ethanol. The *C. longa* extraction was extracted by shaking overnight. The *C. longa* extract solution was filtered and dried at 50°C for 48 hrs. Dimethyl sulfoxide (DMSO, Sigma) was added to dried *Curcuma longa* extract and adjusted the final concentration to 500 mg $mL^{-1}$ before being used.

**Antibiotic-resistant bacteria:** *Proteus mirabilis*, *Acinetobacter baumannii* and multidrug-resistant *Klebsiella pneumoniae* (MDR-K) were obtained from the Department of Clinical Microbiology, Roi Et Hospital, Roi Et, Thailand. A single colony was transferred to 5 mL nutrient broth (NB) and incubated at 37°C with shaking overnight. The cell concentration of fresh antibiotic-resistant bacterial strain was adjusted at $OD_{600}$ to 0.1 before use[13].

**Antibacterial activity determination using the disc diffusion method:** One hundred each antibiotic-resistant bacteria

($OD_{600}$ to 0.1) were spread onto NA. The filter paper with a diameter of 0.6 mm was placed on NA. Ten ethanolic *Curcuma longa* L. extract microliters were dropped onto the paper disc (triplicate). The plate was allowed to diffuse for 15 min and incubated at 37°C for overnight. The zone of inhibition around the disc was measured.

**Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination:** The MIC and MBC values were determined by the microdilution method. One hundred microliters of NB were added to each well and the ethanolic *Curcuma longa* L. extract was twofold serially diluted. One hundred microliters of each antibiotic-resistant bacteria ($OD_{600}$ to 0.1) were added to each well. The plate was incubated at 37°C overnight before MIC and MBC values were measured by colorimetric assay. The iodonitrotetrazolium (INT) was added to each well and waited for 30 min. The wells without bacteria are yellow in color and the wells with bacterial growth turned pink in color. The MIC value indicated the lowest concentration of the ethanolic *Curcuma longa* L. extract that inhibits antibiotic-resistant bacterial growth. The MBC was referred to as the lowest concentration of ethanolic *Curcuma longa* L. extract that eliminates the antibiotic-resistant bacteria[12].

**Statistical analysis:** The data for each inhibition zone was expressed as Mean±Standard error.

## RESULTS AND DISCUSSION

**Disc diffusion method:** The disc diffusion method was used for the primary screening of antibacterial activity. The result indicated that the ethanolic *Curcuma longa* L. extract exhibited antibacterial activity against *P. mirabilis* and MDR-K, except *A. baumannii*. The largest zone of inhibition at 9 mm was obtained from *P. mirabilis* and followed by MDR-K at 8 mm (Fig. 1). The result of the inhibition zone of *Curcuma longa* L. extract from this research was similar to the previous report about the ethanolic extract of *Curcuma longa* at 100, 150 and 200 mg $mL^{-1}$ can inhibit the growth of *K. pneumoniae* with inhibition zone at 13, 17 and 20 mm, respectively[11]. Rattanasuk *et al.*[12] presented the inhibition zone from *Cathormion umbellatum* extracts against *P. mirabilis* and MDR-K at 7 and 11 mm. The extracts from *Litsea glutinosa* L., *Vitex peduncularis* W. and *Elephantopus scaber* L. at 250 µg/disc were presented the inhibition zone against *P. mirabilis* at 11.8±0.27, 13.4±0.2 and 14.5±0.4 mm, respectively[14]. The acetone extracts of *Clitoria ternatea* at 100-500 µg showed the inhibition zone against *P. mirabilis* at 20-26 mm[15]. Hassan *et al.*[16] reported the antibacterial activity of *Curcuma longa* root extract against *P. mirabilis* and *K. pneumoniae* with the zone of inhibition at 8 and 12 mm. Sathishkumar *et al.*[17] presented the curcuminoid has inhibited the growth of *P. mirabilis* and *K. pneumoniae* with inhibition zone at 10 and 9 mm. Ajige *et al.*[18] reported the zones of inhibition from *Curcuma longa* rhizome ethanolic extract (40 g/100 mL) against *P. mirabilis* and *K. pneumoniae* were at 9.89 and 8.00 mm. Elkamali and Mahjoob[19] reported that *Curcuma longa* roots extract inhibited the growth of both *P. mirabilis* and *K. pneumoniae* with an inhibition zone of 4 mm.

**MIC and MBC values:** The INT reagent was used as an indicator in a colorimetric assay for MIC and MBC values determination. After 30 min incubation, the color of the INT

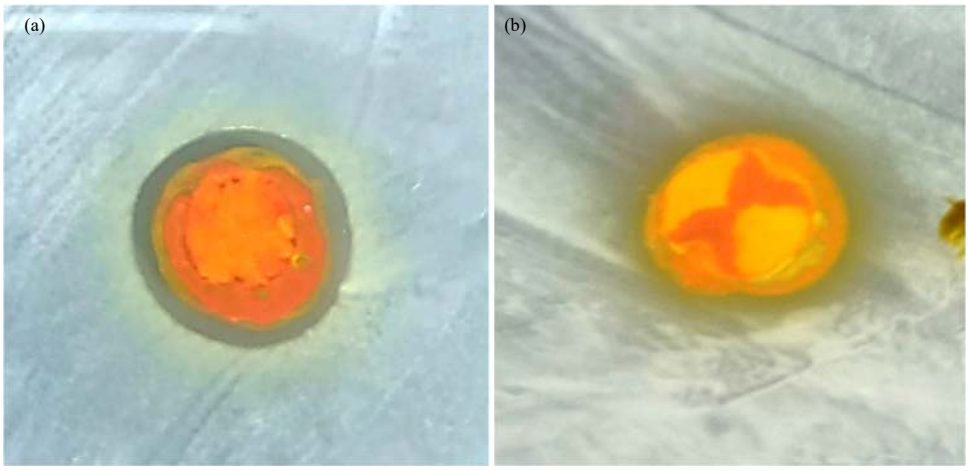


Fig. 1: Inhibition zone of ethanolic *Curcuma longa* L. extract against (a) MDR-K and (b) *Proteus mirabilis*

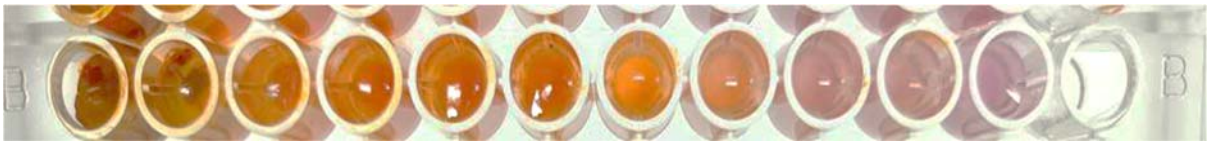

Fig. 2: Colorimetric assay using INT for MIC and MBC values determination

solution indicated the value of MIC and MBC (Fig. 2). The result presented that the MIC value of ethanolic *Curcuma longa* L. extract against *P. mirabilis* and MDR-K were 0.049 and 0.195 mg $mL^{-1}$. The MBC value of ethanolic *Curcuma longa* L. extract against *P. mirabilis* and MDR-K were 0.39 and 6.25 mg $mL^{-1}$. The results from this research were better than some previously reported. Sathishkumar *et al.*[17] reported the *Curcuma longa* L. extract has MIC against *P. mirabilis* and *K. pneumoniae* at 0.250 and 0.350 mg $mL^{-1}$. Ajige *et al.*[18] presented that the MIC of *Curcuma longa* rhizome ethanolic extracted cannot inhibit the growth of *K. pneumoniae*. de Assis *et al.*[20] reported the result of the antibacterial activity of Lamiaceae plant extracts in clinical isolates of multidrug-resistant bacteria. Their results indicated that the crude extracts of the Lamiaceae family against *K. pneumoniae* ranged from 0.5-2 mg $mL^{-1}$ [16]. Rattanasuk *et al.*[12] indicated that the MIC and MBC values of *Cathormion umbellatum* extracts against *P. mirabilis* and MDR-K were ranging from 1.6 -6.3 mg $mL^{-1}$ [12].

*Curcuma longa* has been shown to exhibit antibacterial activity against various strains of bacteria. The active ingredient in turmeric, curcumin, has been found to have potent antibacterial properties against both Gram-positive and Gram-negative bacteria. In addition to its direct antibacterial effects, curcumin has also been shown to have synergistic effects with some antibiotics, enhancing their effectiveness against bacterial strains. This may offer a promising approach to combat antibiotic-resistant bacteria. It's important to note that while there is evidence to support these potential benefits of turmeric and curcumin, more research is needed to fully understand their effects on human health. Additionally, consuming turmeric or curcumin in food or supplement form may not be appropriate for everyone and it's important to consult with a healthcare provider before adding them to your diet or supplement routine.

## CONCLUSION

The *Curcuma longa* L. was extracted with ethanol and was determined the antibacterial activity against *P. mirabilis*, *A. baumannii* and MDR-K. The results indicated that the *Curcuma longa* extract can be inhibited the growth and eliminated both *P. mirabilis* and MDR-K. The new finding of this research presents an antibacterial activity profile of *Curcuma longa* L. against *P. mirabilis* and MDR-K. This result is useful for new antibiotic development against *P. mirabilis* and MDR-K to reduce the death of patients in the hospital.

## SIGNIFICANCE STATEMENT

This study discovers the antibacterial activity of ethanolic *Curcuma longa* L. extracts against three antibiotic-resistant bacteria. This study will help the researcher to uncover the critical areas of using ethanolic *Curcuma longa* L. extracts as a natural antibiotic that many researchers were not able to explore. Thus, a new application using the antibacterial activity of ethanolic *Curcuma longa* L. extracts against three antibiotic-resistant bacteria may be arrived at.

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