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Research Article

ANTIBACTERIAL ACTIVITY OF TWO NATIVE *CURCUMA* SPECIES OF LAO PDR

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A B S T R A C T

Curcuma is the largest genus in the Zingiberaceae and common use as food, spices, herbal medicines, dyes, and beauty treatments. In this study, the antibacterial properties of *Curcuma longa* and *Curcuma aeruginosa*- two popular *Curcuma* species collected from Lao PDR were investigated using agar well diffusion method. Plant crude extract and its fractional n-hexane, methanol, ethylacetate and dichloromethane from *C. aeruginosa* showed strong inhibitory activity against *S. aureus* and *P. aeruginosa* with the MIC values ranging from 53,3 to 180µg/mL. Three of four fractional extracts from *C. longa* (methanol, ethylacetate and dichloromethane) suppressed the development of *S. aureus* while only n-hexane and dichloromethane fractions express similar properties. The MICs values of *C. longa* were in the range of 110 to 2 15µg/mL.

INTRODUCTION

Lao People's Democratic Republic (Lao PDR) is a landlocked country located in South East Asia covered by about 40% of forest (Vongsiharath, 2011) and one of the countries with the richest biodiversityin the region (Phompila *et al*, 2017). It is a mega-diverse country belonging to the Indo-Burma biodiversity hotspot with an estimated 8000-11,000 species of plants and on-going discoveries of new and large species (Soejarto *et al*, 1996; Soejarto *et al*, 1999). Additionally, seventy-three percent of the population lives in rural areas of the country, where access to conventional medicine is limited, therefore the usage of Lao traditional medicine for primary health care is very important, resulting in prevalent traditional healers in rural villages and towns (Soejarto *et al*, 2006; Libman *et al*, 2006).

The number of medical plants is diverse in different areas and communities in Lao PDR (Soejarto *et al*., 2006; Soejarto *et al*, 2009). According to ethnobotany-driven survey of the medicinal plants of Laos through field interviews with healers in 15 provinces and the Vientiane Prefecture, approximately 1000 medical plants were documented. Nearly half of the sample extracts demonstrated *in vitro* activities against bacteria, cancer, HIV/AIDS, tuberculosis and malaria diseases, suggested a huge potential for these plants to exhibit biological activity and provide new chemical constituents that may serve as candidates for medicines (Soejarto *et al*, 2012).

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The genus *Curcuma* is one of the most widespread genera of the Zingiberaceae consisting of more than 80 accepted species, which are nativein tropical Asiaincluding Southeast Asia, southern China, India to New Guinea and northern Australia. In the Checklist of the Vascular Plant of Lao PDR (Newman *et al*, 2007), 15 species of this genus were reported and three new species of *Curcuma peramoena* Souvann. &Maknoi, sp. nov., *Curcuma corniculata* and *Curcumaflammea* (Curcuma subg. Ecomata) have been determined (Leong-Škorničková *et al*, 2014; Souvannakhoummane and Maknoi, 2014). The rhizomes of *Curcuma* are the source of yellow dye and have been used widely as coloring agent and spices all over the world.They are also the major ingredient in folk remedies for the treatment of skin diseases and stomach ulcers (Reddy *et al*, 2009). In some Asian countries, *Curcuma* is well-known for the treatment of enlarged liver, spleen, diabetes, cough, hepatic disorders, chest pain, boils, blood purifier, and rheumatism (Abas *et al*, 2005; Al-Reza *et al*, 2010; Xiang *et al*, 2011; Saikia *et al*, 2010; Devi *et al*, 2014). The components of *Curcuma* consists of starch, carbohydrates, proteins, sugars, fats, vitamins, minerals and terpenoids, sterols, organic acids, fatty acids (Larsen *et al*, 2009; Ahemefula *et al*, 2014). The most well-known bioactive compounds from *Curcuma* are curcuminoids including mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin in which the potent antioxidant and antimicrobial- curcumin is the most important compound (Ammon *et al*, 1991; Maheshwari *et al*, 2006; Hayakawa *et al*, 2011). Recently, six sesquiterpenoids were isolated from a methanol extract of *Curcuma aeruginosa*

rhizomes collected in Champasak, Southern Laos (unpublished data).

The curcumin from *Curcuma longa* L., the widely grown species in *Curcuma* genus have been subjected to a variety of antimicrobial investigations. The methanol extracts of *C. longa* exhibited strong antibacterial activities against *Bacillus subtilis* and *Staphylococcus aureus* with the minimal inhibitory concentration (MIC) values of 16 µg/mL and 128 µg/mL, respectively (Niamsa *et al*, 2009). The hexane and methanol extracts of *C. longa* inhibited the development of 24 pathogenic bacteria isolated from the chicken and shrimpin which 13 bacteria species of the *Vibrio* genus, *Staphylococcus* genus or *Bacillus* genus were strongly inhibited (Lawhavinit *et al*., 2010). Curcumin was found effective against *B. subtilis*, *B. coagulans*, *B. cereus*, *E. coli*, and *P. aeruginosa*, especially the MIC value against *S. aureus* strains was significant in the range of 125-250 µg/mL (Negi *et al*, 1999; Mun *et al*, 2013). Three noval curcumin derivatives from *C. longa* including indium curcumin, indium diacetyl curcumin, and diacetyl curcumin exhibited different inhibitory activity against *S. aureus*, *S. epidermis*, *E. coli*, and *P. aeruginosa* in which indium curcumin had a better inhibitory compared to curcumin itself and other derivatives while diacetylcurcumin did not exhibit any antibacterial effect against tested bacteria (Tajbakhsh *et al*., 2008). Additionally, the *in vitro* evaluation of curcumin against 65 clinical isolates of *Helicobacter pylori*, the pathogenic bacterial usually in stomach revealed the significant antibacterial activity with MIC values between 5 and 50 µg/mL (De *et al*, 2009). Molecular studies on the activation of curcumin showed the inhibitory effect on NF-κB pathway, resulting in the release of IL-8 and cell scattering which led to a reduction in inflammation of gastric tissue as the main consequence for *H. pylori* in stomach (Foryst-Ludwig *et al*, 2004).

In order to evaluate medical value of native *Curcuma longa* and *Curcuma aeruginosa* of Lao PDR, in this study antibacterial activity of such important *Curcuma* species was investigated using crude extracts and different fractions. The obtained results could be a foundation for further discovery of potential bio-compounds from *Curcuma* species in Lao PDR.

MATERIALS AND METHODS

Plant materials

The samples of *Curcuma longa* L. were collected in Phou Khao Khouay National Park, Vientiane province in October 2017 while the ones of *C. aeruginosa* Roxb. were collected at Champasak province in June 2018 and taxonomically classified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). Voucher specimens were deposited at the Institute of Marine Biochemistry, VAST, Hanoi, Vietnam and Laboratory of Life Sciences, Laos Academy of Science, Lao PDR.

Preparation of total extract and fractions

Dried rhizomes (1.0 kg) of both species were powered and extracted in 10L of methanol successively three times for 1 h, each time using ultrasonic assisted extraction method. The temperature of sonicator was fixed to 40℃ with power 170 W and frequency 42 KHz for 15 minutesto get maximumyield. The extracts were recovered by filtration through Whatman filterpaper No.1. The methanol extracts was suspended in 2.0 L water and partitioned in turn with ethylacetate (3×2.0 L), n-hexane (3×2.0 L) and dichloromethane (3×2.0 L) to obtain ethylacetate (E), n-hexane (H) and dichloromethane (D) fractions and water layer (W). The fractions obtained were evaporated to dryness by a rotary evaporator and stored in well closed containers under refrigeration conditions and dilutions of the plant extract and fractions in DMSO were used for antimicrobial studies.

Microorganism strains and culture

Five Gram negative pathogenic bacteria strains (*Escherichia coli* ATCC® 25922™*, *Salmonella typhimurium* ATCC®19430™, *Proteus mirabilis* ATCC®29245™*, *Proteus vulgaris* ATCC® 33420™*, *Pseudomonas aeruginosa* ATCC® 15692™*) and two Gram positive strains (*Staphylococcus aureus* ATCC® 25923™, *Bacillus subtilis* ATCC® 6633™) were procured from Microbiologics (America). The bacterial strains were maintained on LB medium at 4°C and subcultured on a fresh agar plates 24h prior to any antibacterial test. Culture media were sterilized by autoclaving at 121°C for 20 minutes.

Agar well diffusion method

The total and fractional extracts from *Curcuma* rhizomes were subjected to antimicrobial assay using the agar well diffusion method as described by Tran *et al* (2013). Extracts were diluted to 25, 50, 100, 200 and 300mg/mL in DMSO before testing. Gentamycin and DMSO were used as positive and negative controls respectively. The antimicrobial activity was evaluated after 24hours and inhibition zone value (IZ) were measured in which IZ (mm) = D - d (D: diameter of inhibition zone, d: diameter of well). The experiments were done in triplicate and the average values were recorded for evaluation. The inhibition of the organism on medium containing the test compound was also compared with the inhibition on the plates containing antibiotic gentamyc in as standard.

Minimum Inhibitory Concentration (MIC)

MIC was determined using broth dilution method. The extracts were diluted in LB medium to give the final concentrations of 0.2-8.0 mg/mL depending on the antibacterial activity from agar well diffusion assay before. About 5 µl of 10^5 CFU/ml of the bacterial strains was inoculated in 3mL of each dilution at 37°C for 24 hours. Positive control is the tube containing Gentamycin diluted at 0.1mg/mL in LB medium and negative controls were different diluted plant extracts without bacteria. The growth of bacteria was determined at OD_{600}. The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24h when compared with the control tubes as considered as MIC.

RESULTS

Evaluation of antibacterial activity

Methanol total extract (M), and its fractions including ethylacetate (E), n-hexane (H), dichloromethane (D) and water layer (W) were isolated from two *Curcuma* species and used for antibacterial tests against seven bacterial species by agar well diffusion method. The obtained results indicated the inhibition zone of gentamyc in was ranged from 10 to 20mm, depending on the microbial tested. No inhibition zone was observed in wells containing negative control (DMSO), meaning the positive and negative controls are comparable.

Apart from water layers, which did not show any antibacterial activities, plant crude extracts and their fractions all possessed antibacterial activities in different levels depending the bacterial species, plant species, crude extracts and fractions. Among tested bacteria, the growth of *S. aureus* and *P. aeruginosa* are the most impact by plant extracts and different fractions. In general, *C. aeruginos a* total extract and its fractions gave the larger inhibition zones against both *S. Aureus* and *P. aeruginosa* than *C. longa*. N-hexane fraction of *C. aeruginosa* at concentration of 100mg/mL strongly suppressed the development of *S. aureus,* providing the maximum inhibition zone of 20mm, similar to the positive control (0.1mg/mL Gentamycin), while n-hexane fraction of *C. longa* did not show any antibacterial activity against *P.aeruginosa* (Fig. 1). Similarly, the anti *P. aeruginosa* activity observed for methanol extract, n-hexane, dichloromethane and ethylacetate fractions from *C. aeruginosa* in which n-hexane exhibited the highest activity of 21.3 mm, significant higher than the positive control (Fig. 2).

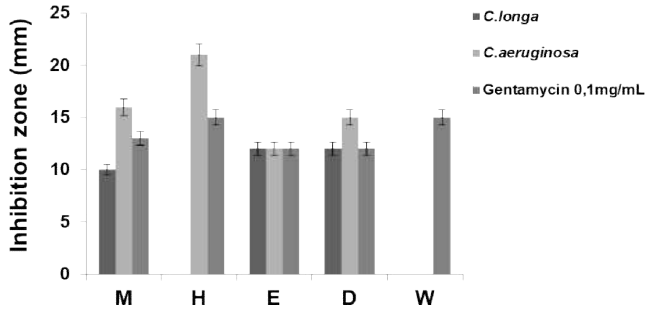

Figure 1 Antibacterial activity against *S. aureus* of *C. longa* and *C. aeruginosa*

The inhibition zone measured in mm, mean of triplicates. Gentamycin (0,1 mg/mL) was used as positive control. M, H, E, D, W are fractional extracts of methanol, n-hexane, ethylacetate, dichloromethane and water layer.

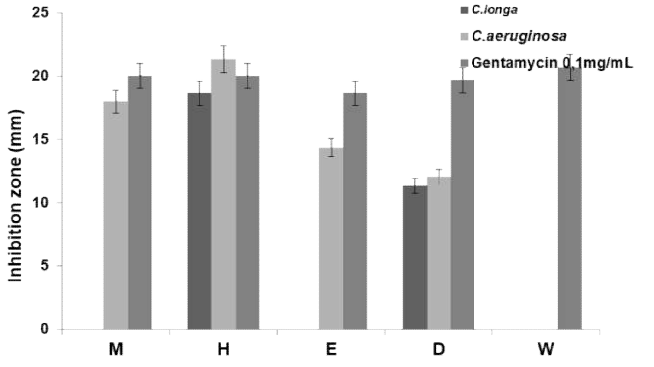

Figure 2 Antibacterial activity against *P. aeruginosa* of *C. longa* and *C. aeruginosa*

The inhibition zone measured in mm, mean of triplicates. Gentamycin (0,1 mg/mL) was used as positive control. M, H, E, D, W are fractional extracts of methanol, n-hexane, ethylacetate, dichloromethane and water layer.

Only n-hexane and dichloromethane fractions at 100mg/mL of *C. longa* showed anti -*P.aeruginosa* activity with the obtained inhibition zones at 18.6mm and 11.3mm, respectively.

The antibacterial activity of plant extracts and their fraction from rhizomes of both *C. longa* and *C. aeruginosa* against either Gram negative strains of *E. coli*, *S. typhimurium*, *P. mirabilis*, *P. vulgaris* or Gram positive strain of *B. subtilis* were detectable at concentrations lower than 100mg/mL. Moderate activity against *B. subtilis* and *E. coli* can be observed using 200-300mg/mL of methanol extracts from *C. aeruginosa*. However, there was no significant difference in antibacterial activity against two bacterial strains at concentrations of 150, 200 and 300mg/mL of *C. longa* with the inhibition zones ranging from 2 to 4mm. The growth of *E. coli* or *B. subtilis* was inhibited weakly by 300mg of extracts from *C. longa* expressing by the inhibition zone was 1-2mm approximate 5-10% of positive control.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is defined as the lowest concentration at which no visible bacterial growth is observed. MIC value of total extracts and their fractions of *C. longa* and *C. aeruginosa* were determined using broth dilution method. Values for MICs were dependent on bacterial species and testing extracts and fractions. Based on the results of agar diffusion assay, MIC values were evaluated in methanol extracts, n-hexane, dichloromethane and ethylacetate fractions against two bacterial strains of *S. aureus* and *P. aeruginosa*. The MIC values revealed that *C. aeruginosa* exhibited the antibacterial properties stronger than *C. longa*. As shown in table 1, n-hexane fraction from *C. aeruginosa* was the most potent against both *S. aureus* and *P. aeruginosa* with the MIC values around 50µg/mL. Dichloromethane fractions from these *Curcuma* species also showed strong inhibitory activity against *P. aeruginosa* with the MIC value of 55µg/mL. The lowest MIC value was obtained by the n-hexane fraction at concentration of 110.0µg/mL of *C. longa* against *P. aeruginosa*. MIC values of other fractions were higher from 183 to 215µg/mL (Table 1)

Table 1 MIC values of fractional extracts from*C. longa* and *C.aeruginosa*

Extract	MIC (µg/mL)			
	C. longa		*C. aeruginosa*	
	S. aureus	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*
Methanol	215 ± 15	-	160.0 ± 11.3	96.7 ± 5.7
n-hexane	-	110.0 ± 5.0	56.7 ± 5.8	53.3 ± 5.7
Ethylacetate	186.7 ± 11.5	-	180.0 ± 10.0	110.0 ± 0.0
Dichloromethane	196.7 ± 5.8	183.0 ± 5.7	163.3 ± 15.2	55.0 ± 5.0

DISCUSSION

The benefit of herb-derived drugs was known worldwide for thousand years and still plays the pivotal role in the pharmaceutical industry. Up to date, 80% of the population in developing countries of Asia and Africa depends on traditional medicine for primary health care (WHO Traditional Medicine Strategy 2002-2005). Chemical analysis of many medical plants has shown the presence of various bioactive and/or new compounds explaining the effectiveness of these plants in disease treatment. Being a hot spot of biodiversity, Lao PDR possesses the capability to investigate and develop the bioactive compounds from plant natural resources for medicinal properties. Therefore, it is important to determine the antibacterial activity of two native *Curcuma* species in Laos for the further isolation bio-active compounds from these species, providing scientific background and efficient methods for Laboratory of Life Sciences, Laos Academy of Sciences to evaluate to natural resources in Laos.

In this study, crude extracts and their fractions of *C. longa* and *C. aeruginosa* collected from Lao PDR were evaluated for their antibacterial activity by disc diffusion method against seven pathogenic bacteria. All of them were found to be effective against these micro-organisms, especially *S. aureus* and *P. aeruginosa*. *S. aureus* is the Gram positive bacteria frequently found in the nose, respiratory tract and on the skin.

S. aureus can cause skin diseases including abscesses, respiratory infections such as sinusitis, and food poisoning (Tong *et al*, 2015) while *P. aeruginosa*, a Gram negative bacteria typically infects the airway, urinary tract, burns, and wounds (Balcht and Smith 1994). The effective of Curcuma extracts against pathogenic bacteria could be a consequence of morphological deformity, with partial lack of the cytoplasmic membrane, leading to cell disruption, which already reported by Gupta *et al* (2015). Our findings could provide addition scientific background for the use of *Curcuma* to treat common eye infections, to dress wounds, treat various skin diseases, bites or acne, and effective in recovery the stomachache from ancient to up to date.

Total extract, n-hexane, ethylacetate and dichloromethane fractions from *C. aeruginosa* exhibited the strong antibacterial activity against both *S. aureus* and *P. aeruginosa*. In opposite, total methanol extract, n-hexane and an ethylacetate fraction from *C. longa* could not inhibit the development of *S. aureus* or *P. aeruginosa*. N-hexane fraction of *C. aeruginosa* was found to be most effective with the inhibition zone value reached 21mm at concentration of 100 mg/mL. The obtained results showed the strong biological activities of Curcumaspices of Lao PDR. Similarly, when testing antibacterial activity of turmeric extract against ten different bacterial strains using agar well diffusion method Selvam *et al* (2012) also found that a good inhibitory activity against *E.coli* and *Vibrio cholera* with a zone of inhibition 7 to 15mm and 10 to 15mm, respectively.

MIC of *C. aeruginosa* extract was found to be lowest followed by *C. longa*. While the MIC from *C.aeruginosa* extracts were ranging from 53.3 to 180µg/mL, the MIC of *C. longa* ones were higher, from 110 to 215µg/mL. Above observations was previously reported by the study of Wilson *et al* (2005) who evaluated antimicrobial activity of extracts of other Curcuma species *C. zedoaria* and *C. malabarica* tubers against six bacterial strains. The MIC values for different strains and extracts ranged from 10µg to150µg/mL in *C. zedoaria* and from 10 to 940 mg/ml in *C. malabarica*. *S. aureus* was inhibited by *C. malabarica* but not by *C. zedoaria.* Suvarna *et al* (2014) evaluated antibacterial activity of ethanol fraction of *C. longa* powder against *Enterococcus faecalis* and found that MIC of turmeric extract for *E. faecalis* wasn ot established because it did not show any clear zones around the discs at all tested concentrations. The result from this study supported the use of *Curcuma* for antimicrobial treatment diseases, especially in skin or wound.

CONCLUSION

Our study demonstrated the antibacterial activity of two *Curcuma* species collected in Lao PDR. The crude extracts and their fractions of *C. longa* and *C. aeruginosa* exhibited strong antibacterial properties and could be a value natural source for isolating antibiotic compounds against *S. aureus* and *P. aeruginosa*. In near future, the chemical components in each extract and their activities must be further carried out to sustainable apply the indigenous knowledge to modern pharmaceutical purpose.

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