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ANTI-TYROSINASE, ANTI-SKIN PATHOGENIC BACTERIAL, AND ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL CONSTITUENTS OF *Dracaena cochinchinensis* (Lour.) S.C. Chen EXTRACT

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Abstract

Dracaena cochinchinensis (Lour.) S.C. Chen (Chandaeng) is an important traditional medicinal plant used in ancient Thai household remedies. This research focused on investigating the biological properties, including the antibacterial, anti-tyrosinase, antioxidant activities, and phytochemical characteristics of crude Chandaeng extracts. Dried Chandaeng heartwood powder was extracted using ethanol, methanol, and deionized water. The antibacterial activities of the extracts were then tested against skin pathogens, including *Cutibacterium acnes* (DMST14916), *Staphylococcus epidermidis* (TISTR518), and *Staphylococcus aureus* (TISTR321). The ethanolic extract showed antibacterial activity. In a time-kill assay, all bacteria were completely killed after being exposed to it, while the cell membranes were found to have leaked when viewed under a scanning electron microscope. Antioxidant potential was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis -3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. According to the findings, the crude ethanolic extract of Chandaeng showed the highest level of antioxidant activity. Furthermore, the potential of the extract to treat skin hyperpigmentation by inhibiting tyrosinase, an important melanin synthesis enzyme, was determined and the ethanolic extract was found to be an anti-tyrosinase agent. Finally, the crude ethanolic extract showed the highest total phenolic compound and flavonoid content. In conclusion, crude Chandaeng extract showed significant potential in activity against skin pathogenic bacteria, antioxidant activity, and tyrosinase inhibition. These properties of the extract could be applied to skincare cosmetics.

Keywords: Antibacterial. Antioxidant. *Dracaena cochinchinensis* (Lour.) S.C. Chen. Tyrosinase inhibition.

1. Introduction

Most people experience skin conditions such as allergies, dermatitis, infections, and acne. The important causes of acne involve both endogenous and exogenous factors such as hormonal changes, genetic material, stress, and microorganism infections of the skin (Davis et al. 2010). The skin of those who have inflammatory acne is contaminated with bacteria that cause acne, such as *Staphylococcus epidermidis, Staphylococcus aureus*, and *Cutibacterium acnes* (Hassanzadeh et al. 2008). However, these bacteria also have drug-resistant variants mainly to the widespread administration of antibiotics as medical therapies. Therefore, a natural product made from herbal extracts or medicinal plants is a treatment alternative that can help to reduce antimicrobial resistance (Rahman et al. 2016).

Melanin buildup in the layer of the skin, which results in skin pigmentation or melanogenesis and might be undesired, is another intriguing skin issue. The dark pigment melanin, which is present in both hair and skin, is crucial for shielding human skin from radiation. Melanin pigments are created during the physiological process of melanogenesis, which also protects against UV-induced skin damage and helps prevent skin cancer (Robb 1984). Tyrosinase (EC 1.14.18.1; PPO) is an important enzyme in the production of melanin (Ferrer et al. 1995). Therefore, researchers and scientists have been encouraged to research on the finding, extraction, biosynthesis, and characterization of novel powerful tyrosinase inhibitors from plants and medicinal herbs for use in formulating cosmetic products for skin whitening (Kadekaro et al. 2003). Both whitening agents in cosmetic applications and nutritional sources have been developed from natural compounds derived from plants. Herbal extracts with antioxidant properties are of particular interest because they perform a variety of beneficial activities, including preventing the formation of free radicals, reducing UV radiation-mediated oxidative damage by preventing the spread of oxidizing chain reactions, as well as inhibiting tyrosinase activity (Ribeiro et al. 2015).

Dracaena cochinchinensis (Lour.) S.C. Chen is also known in Thai as Chandaeng, Chanpha, or Lukka-chan. Chandaeng is one of the medicinal plants used in ancient Thai household remedies. It is included in the list of herbal medicines and is frequently used to treat illnesses caused by infections. In limestone regions of steep mountains, Chandaeng is typically found on sunny cliffs, and the resin collected from its stems is a source of “Dragon’s Blood”. These resinous drugs are frequently recommended in China to stimulate blood flow in the treatment of pain, blood stasis, and severe injuries (Fan et al. 2014). Chandaeng contains important phytochemical elements such as loureirin A, loureirin B, loureirin C, cochinchinenin, socotrin-4'-ol, 4',7-dihydroxyflavan, 4-methylcholest-7-ene-3-ol, ethylparaben, resveratrol, and hydroxyphenol. These compounds exhibited anti-bacterial, anti-fungal, antioxidant and anti-inflammatory activities (Pang et al. 2021; Wang et al., 1995). *S. aureus*, *Diphtheria bacilli*, and *Bacillus anthracis* are all inhibited by Chandaeng extract, as previously described (Cai et al. 1990). In addition, the compound 2,4-dihydroxy benzaldehyde from Chandaeng exhibits substantial antifungal effects against the molds *Penicillium digitatum*, *Botrytis cinerea*, *Magnaporthe grisea*, and *Sclerotinia sclerotiorum* (He et al. 2021). Nevertheless, no report has demonstrated the anti-acne-causing bacterial and anti-tyrosinase activity of crude Chandaeng extract or its applications in skincare or cosmetic products. This research investigated the biological properties of the crude Chandaeng extract used in skincare applications for anti-skin pathogenic bacterial and anti-tyrosinase activities. We also investigated the antioxidant activity and phytochemical contents of crude Chandaeng extract.

2. Material and Methods

Plant material and authentication

Dried Chandaeng heartwood was purchased from a herbal drug store in Nakhon Pathom Province, Thailand. An authenticated voucher specimen of *Dracaena cochinchinensis* (Lour.) S. C. Chen (T. Soingam 04101801) was deposited at the Department for Development of Thai Traditional and Alternative Medicine.

Maceration

The ground sample (10 g) was weighed into two separate conical flasks containing 100 mL of ethanol and methanol, respectively, then shaken in an orbital shaker (New Brunswick Scientific, USA) for 72 h. Hot extraction of Chandaeng powder (10 g) in deionized water (DI, 100 mL) was also performed. Whatman No. 1 filter paper was used to filter the crude extracts, and a rotary evaporator was then used to further concentrate the filtrates till they were dry (Heidolph, Germany). The yield (% w/w) of each extract was determined, and the samples were kept at -20 °C until use (Gupta et al. 2013).

Biological activity

Antibacterial activity

The agar well diffusion technique was used to test antibacterial activity. The turbidity of an inoculum suspension including *C. acnes* DMST14916, *S. epidermidis* TISTR518, and *S. aureus* TISTR321was adjusted to equal the turbidity of 0.5 McFarland standards (10^8 CFU/mL). About 100 µl of the test microorganisms were spread over the entire surface of the agar. On each plate, three equidistant wells (8 mm in diameter) were made with a sterilized cork borer. Then, 150 µl of Chandaeng extract (50 mg/mL) was added to each well. The plates were incubated for 24–72 h at 37 °C. The inhibition zones that appeared on the plate were measured. All extraction solvents were used as negative controls. Antibiotic discs, containing Ampicillin, Clindamycin, and Tetracycline, were used as positive controls. The samples were tested in triplicate (CLSI 2012a).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC values were obtained using the two-fold broth dilution technique (CLSI 2012b). Crude Chandaeng extract was serially diluted with broth medium to give final concentrations of 0.781–25 mg/mL. One milliliter of crude Chandaeng extract was mixed with 1 mL of inoculum suspension to achieve a final turbidity equivalent to the 0.5 McFarland standards and incubated at 37 °C for 24 h. The tube containing medium-tested inoculum and extraction solvent served as a control. The lowest dilution of the tube that revealed no turbidity was considered to be the MIC value. The MBC was the lowest concentration that killed the whole bacteria on the plates. Each test was performed in triplicate.

Time-kill assay

Culture samples of *C. acnes* DMST14916, *S. epidermidis* TISTR518, and *S. aureus* TISTR321 cells (0.5 McFarland standards) were treated with crude ethanolic Chandaeng extract diluted with broth media containing inoculum to obtain final concentrations of 0xMIC, 1xMIC, 2xMIC, and 4xMIC for each bacterial species. Cultures were incubated at 37 °C and 150 rpm. At a specified time (0, 0.5, 1, 2, and 4 h), 100 µL of aliquots were serially diluted. After incubation at 37 °C for 24–72 h, the number of colonies appearing on the plate was counted. The time-killing activity of the extract was reported as a log (CFU/mL). Samples were analyzed in triplicate (Rahman et al. 2016).

Scanning electron microscope (SEM)

Culture samples of *S. aureus* TISTR321, *S. epidermidis* TISTR518, and *C. acnes* DMST14916 cells were treated with crude ethanolic Chandaeng extract at 1xMIC (3.125 mg/mL) for 2 h, 2xMIC (6.25 mg/mL) for 2 h, and 2xMIC (6.25 mg/mL) for 4 h, respectively. Cultures were incubated at 37 °C. Then, the cells were centrifuged at 6,000 rpm for 10 min. The precipitated cells were rinsed with 0.1 mol L^{-1} phosphate-buffered saline (PBS) at pH 7.4, and then fixed with 2.5% glutaraldehyde for 2 h at 4 °C. Cells were dehydrated in an ethanol series (30%, 50%, 70%, 80%, and 90%) for 15 min at each graduation after a quick washing in PBS and followed by 100% ethanol twice for 15 min. After air drying, the cells were coated with carbon and gold. Scanning electron microscopy (Tescan Mira 3, Czech Republic) was used to evaluate the final samples (Chen et al. 2018).

Tyrosinase enzyme assay

The dopachrome technique was used to evaluate tyrosinase activity. Crude Chandaeng extract (100–500 µg/mL) and positive control of Kojic acid (20-100 µg/mL) were observed at a wavelength of 492

nm. The inhibition of tyrosinase was reported as the IC_{50} value. Samples were analyzed in triplicate (Arung et al. 2006).

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl assay (DPPH)

Radical scavenging activity using DPPH was assessed according to the method described by Sakunpak et al. (2012). Crude Chandaeng extract and standards of ascorbic acid, gallic acid, and Trolox (0.0061–50 mg/mL) were observed at a wavelength of 517 nm (Thermo Scientific, USA). The antioxidant capacity of the extracts was reported as the IC_{50} value. Samples were analyzed in triplicate (Sakunpak et al. 2012).

2, 2′-azino-bis -3-ethylbenzothiazoline-6-sulfonic acid assay (ABTS)

Radical scavenging activity using ABTS was tested according to the techniques described by Re et al. The effects of the crude Chandaeng extract and the Trolox standard (0.0061–50 mg/mL) were observed at a wavelength of 734 nm. The antioxidant activity of each extract was expressed as the IC_{50} value. Samples were analyzed in triplicate (Re et al. 1999).

Preliminary phytochemical screening

Standard procedures were used to conduct phytochemical screening of crude Chandaeng extract for the presence of anthraquinones, terpenoids, flavonoids, phenolic compounds, saponins, tannins, and cardiac glycosides (Trease et al. 2002).

Total phenolic assay

Total phenolic compound content was determined using the Folin-Ciocalteu assay. Crude Chandaeng extract (0.1953 mg/mL) and gallic acid standard (20–100 ug/mL) were observed at a wavelength of 765 nm. The total phenolic component content of the extract was quantified as mg gallic acid equivalents (GAE)/g dry weight of the crude extract. The samples were examined in triplicate (Marinova et al. 2005).

Total flavonoid assay

The aluminum chloride colorimetric technique was used to measure the total flavonoid content. Crude Chandaeng extract (0.3906 mg/mL) and a catechin standard (20–100 ug/mL) were observed at a wavelength of 510 nm. The total flavonoid content of the extract was reported as mg catechin equivalents (CE)/g dry weight of the crude extract. Samples were analyzed in triplicate (Dudonne et al. 2009).

Determination of loureirin B

Determination of loureirin B by high-performance liquid chromatography (HPLC) was performed at 40 °C on an Agilent 1200 series HPLC system (Agilent Technologies, USA) consisting of a degasser, pump, auto-injector, column compartment, diode array detector, and a ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm) using a mixture of acetonitrile: 0.4% phosphoric acid in water (40:60) as the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 10 µL of crude ethanolic Chandaeng extract (1 mg/mL); the detection wavelength was 275 nm. The linear regression equation from the calibration graph was used for the quantitative measurement of loureirin B in the concentration range of 0.0125-0.0500 mg/mL (Wang et al. 2011).

Statistical analysis

Each test was performed in triplicate using the same sample. Values were reported as means ± standard deviations (SD). Using IBM SPSS version 16.0 for Windows, data were analyzed using one-way ANOVA followed by Tukey's test ($p < 0.05$).

3. Results

Yield extraction

The highest yield of crude Chandaeng extract was 12.34±2.17% (w/w), obtained using the ethanol extraction method. The yields obtained using the methanol and DI extraction methods were 11.65±3.09% (w/w) and 1.97±0.04% (w/w), respectively.

Antibacterial activity of the crude extract

The results of the antibacterial activity study are shown in Table 1. The crude ethanolic extract of Chandaeng produced the largest inhibition zones against *C. acnes* DMST14916, *S. epidermidis* TISTR518, and *S. aureus* TISTR321 at 27.00 ± 2.65 mm, 26.83 ± 2.51 mm, and 25.33 ± 0.76 mm, respectively. The crude methanolic Chandaeng extract produced inhibition zones of 24.67 ± 0.58 mm, 23.67 ± 1.25 mm, and 19.00 ± 1.00 mm when tested with the three bacterial strains, respectively. Finally, the crude DI extract of Chandaeng produced inhibition zones of 22.67 ± 0.58 mm, 24.50 ± 0.00 mm and 15.33 ± 0.58 mm, respectively. The investigation of the MIC and MBC values of the extracts against *C. acnes* DMST14916, *S. epidermidis* TISTR518, and *S. aureus* TISTR321 revealed that the methanolic and ethanolic extracts showed the strongest inhibition against the tested strains at MIC values of 3.125 mg/mL and MBC values of 6.25 mg/mL. Chandaeng extracted with DI had the lowest antibacterial activities with MIC and MBC values at >25 mg/mL. The time-kill assay showed that the crude ethanolic extract of Chandaeng killed *S. aureus* TISTR321, *S. epidermidis* TISTR518, and *C. acnes* DMST14916 at 4× MIC for 0.5 h (Figures 1A, 1B, and 1C). The populations of *S. epidermidis* TISTR518 and *C. acnes* DMST14916 also showed a reduction of >3 log10 CFU/mL when treated with the extract at 2× MIC for 2 h, as shown in Figures 1B and 1C, but *S. aureus* TISTR321 was completely killed, as shown in Figure 1A.

Table 1. Antimicrobial activity of the extracts.

Samples	Extraction solvent and antibiotic	Zone of inhibition (mm) ± SD		
		S. aureus TISTR321	*S. epidermidis* TISTR518	*C. acnes* DMST14916
Chandaeng	Methanol	19.00±1.00^{b}	23.67±1.25^{a}	24.67±0.58^{ab}
	Ethanol	25.33±0.76^{c}	26.83±2.51^{c}	27.00±2.65^{ab}
	DI	15.33±0.58^{a}	24.50±0.00^{ab}	22.67±0.58^{a}
Positive control	Ampicillin	18.50±0.50^{b}	23.17±0.57^{a}	31.00±7.21^{b}
	Clindamycin	27.33±1.52^{d}	25.67±0.57^{bc}	52.67±0.50^{c}
	Tetracycline	27.00±1.32^{cd}	25.50±1.32^{bc}	71.00±1.73^{d}

Mean values of the inhibition zones in the same column followed by a different letter were significantly different according to SPSS 16.0 by One-Way ANOVA (Tukey's test) at $p < 0.05$.

Scanning electron microscope (SEM)

The untreated *S. aureus* TISTR321 (Figure 2A) and *S. epidermidis* TISTR518 (Figure 2C) showed a grape-like cluster morphology and tightly packed smooth cocci undergoing cell division. After 2 h of treatment with crude Chandaeng extract, cell disruption was observed, caused by the release of intracellular material and cytoplasm (Figure 2B and 2D). The untreated *C. acnes* DMST14916 showed normal rod-like structure and a smooth surface (Figure 2E). However, after 4 h of treatment with crude Chandaeng extract, cells appeared to be damaged, with some irregular surfaces, and the cells were ruptured and shrunken (Figure 2F).

Tyrosinase inhibitory assay

All of the crude Chandaeng extracts inhibited tyrosinase activity in a dose-dependent manner. The ethanolic extract demonstrated the most potent inhibitory activity with an IC_{50} of 98.11 ± 10.03 µg/mL (Table 2). The methanolic and DI extracts had IC_{50} values of 100.17 ± 11.76 and 347.01 ± 5.26 µg/mL, respectively. Kojic acid was used as a standard with an IC_{50} value of 36.82 ± 1.75 µg/mL.

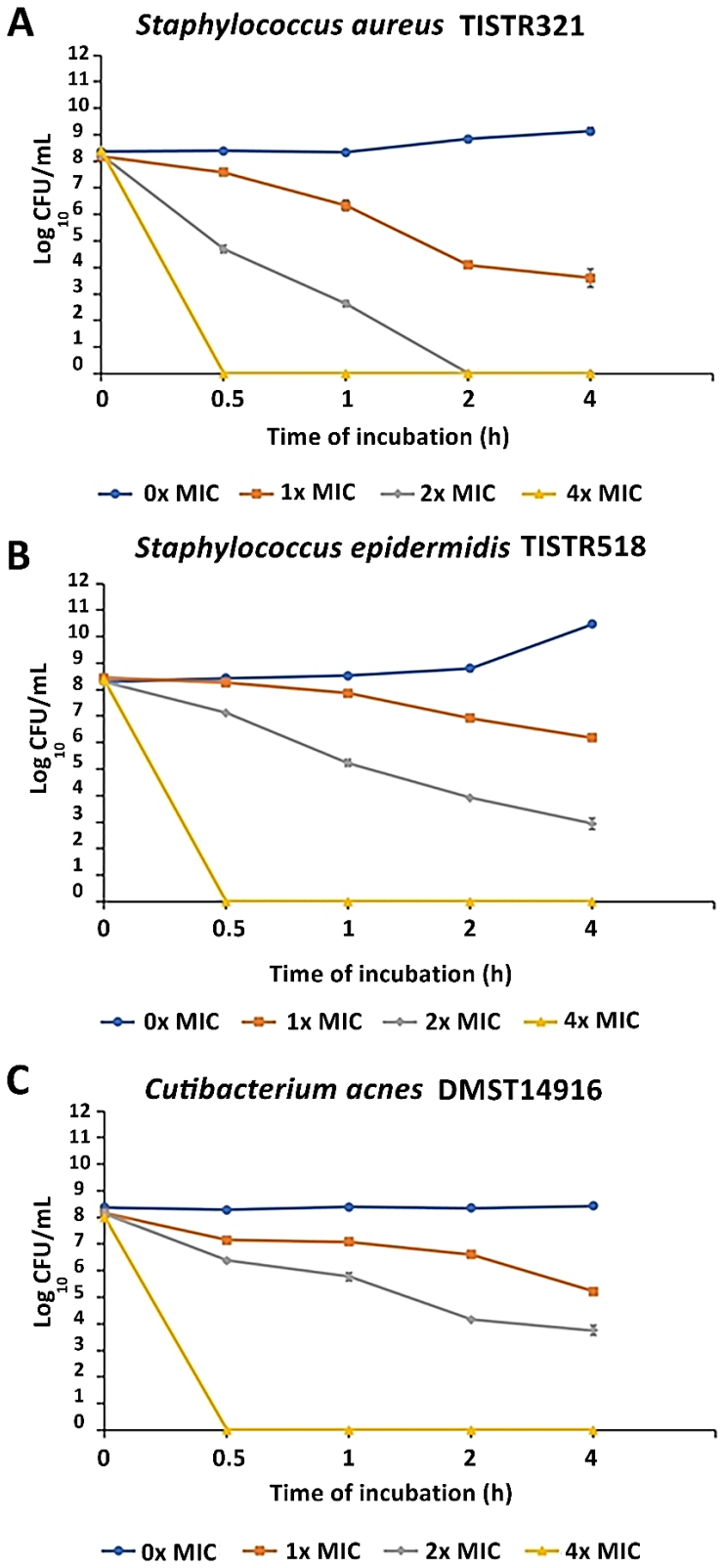

Figure 1. Time-kill curves for *S. aureus* TISTR321 (A), *S. epidermidis* TISTR518 (B), and *C. acnes* DMST14916 (C) after exposure to crude Chandaeng extracts at 0xMIC (Control), 1xMIC (3.12 mg/mL), 2xMIC (6.25 mg/mL) and 4xMIC (12.5 mg/mL).

Table 2. Tyrosinase inhibitory activities of crude Chandaeng extract

Crude Chandaeng extract samples	IC_{50} (µg/mL)	R-Squared
Methanol	100.17±11.76[b]	0.9632
Ethanol	98.11±10.03[b]	0.9914
DI	347.01±5.26[c]	0.9860
Kojic acid	36.82±1.75[a]	0.9782

Mean values with a different letter in the same column were significantly different at the level $p < 0.05$, according to SPSS 16.0 by One-Way ANOVA (Tukey's test).

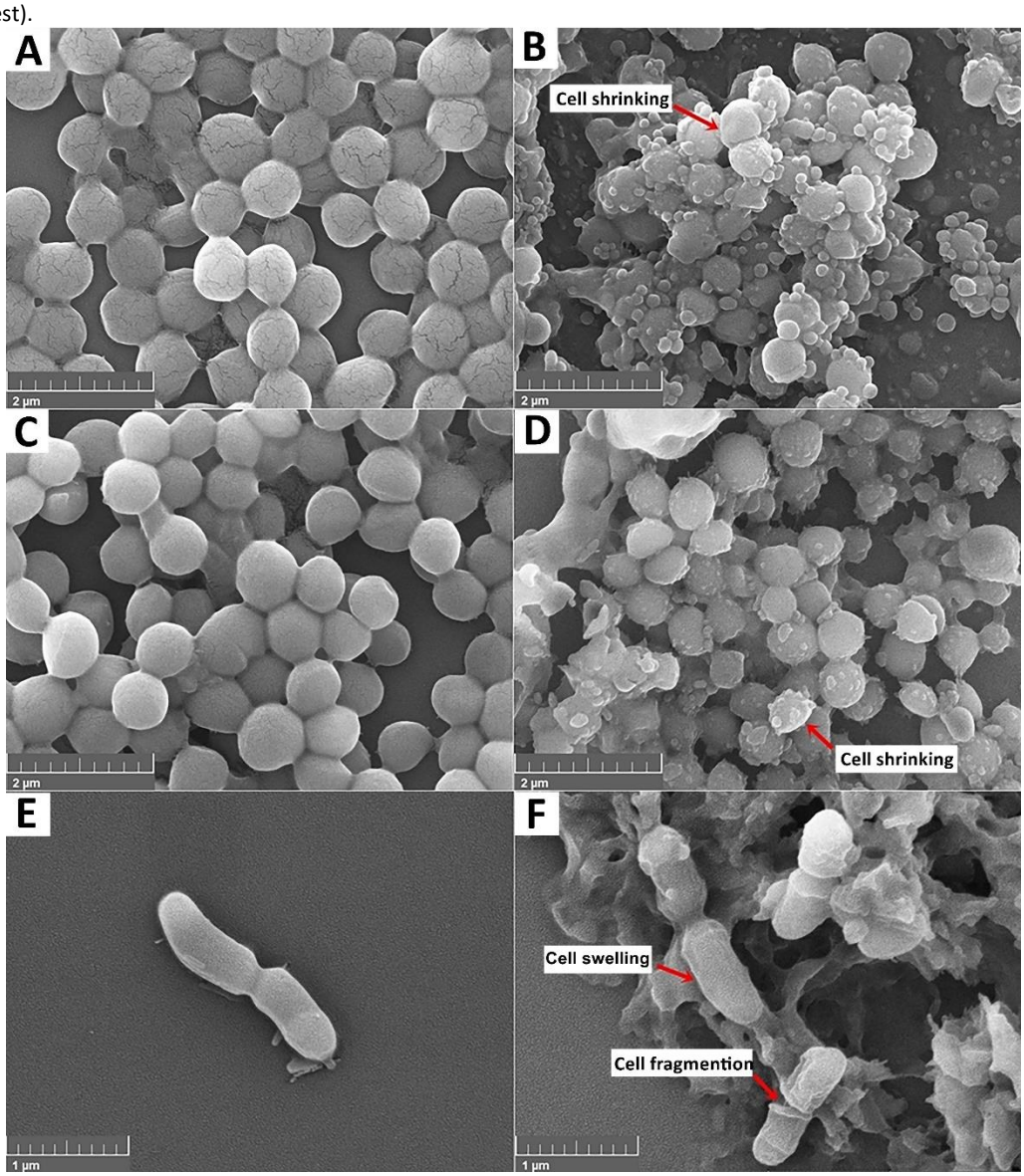

Figure 2. Scanning electron micrographs of untreated *S. aureus* TISTR321 (A) and after treatments with crude Chandaeng extracts at 1xMIC (3.12 mg/mL) for 2 h (B); untreated *S. epidermidis* TISTR518 (C) and after treatments with crude Chandaeng extracts at 2xMIC (6.25 mg/mL) for 2 h (D); untreated *C. acnes* DMST14916 (E) and after treatment with crude Chandaeng extracts at 2xMIC (6.25 mg/mL) for 4 h (F).

Determination of antioxidant activity

The DPPH free radical scavenging assay revealed the ethanolic extract to have the most potent activity with an IC_{50} value of 70.30 ± 0.80 µg/mL, which was higher than those of the methanolic (IC_{50} at 73.10 ± 1.00 µg/mL) and DI (IC_{50} at 107.80 ± 4.40 µg/mL) extracts. In addition, the scavenging activities of the standard compounds, including ascorbic acid, gallic acid, and Trolox showed IC_{50} values of 7.80 ± 0.30, 3.50 ± 0.20, and 6.10 ± 1.90 µg/mL, respectively. The ethanolic extract showed the highest free radical scavenging activity (100.00 ± 0.00%) at a concentration of 3.125 mg/mL, followed by the methanolic extract (97.65 ± 0.72%), while the DI crude extract (75.26 ± 1.72%) showed the lowest scavenging activity. The ABTS radical scavenging assay revealed the ethanolic extract to have the most potent activity with an IC_{50} value of 0.5783 ± 0.0411 mg/mL, while the IC_{50} values using the methanolic and DI extracts were

0.6612 ± 0.0712 and 2.5515 ± 0.1452 mg/mL, respectively. However, a standard compound (Trolox) showed the highest antioxidant activity (IC_{50} = 0.1868 ± 0.0039 mg/mL).

Chemical composition

Preliminary phytochemical screening

Terpenoids, flavonoids, phenolic compounds, saponins, and tannins were found in all crude Chandaeng extracts.

Total phenolic contents

Total phenolic contents were determined using the standard gallic acid (y = 0.0116x, R2 = 0.9988). The crude ethanolic Chandaeng extract contained the highest total concentration of phenolic compounds at 357.35 ± 0.85 mg GAE/g dry weight of the crude extract. The methanolic and DI extracts contained phenolic contents of 356.05 ± 1.17 and 120.31 ± 0.24 mg GAE/g dry weight of crude extract, respectively (Table 3).

Total flavonoid contents

Total flavonoid contents were calculated based on the standard catechin (y = 0.0039x, R2 = 0.9862). The crude ethanolic Chandaeng extract contained the highest total concentration of flavonoids at 667.39 ± 40.11 mg catechin equivalent/g dry weight of the crude extract. The methanolic and DI extracts contained total flavonoid contents of 492.34 ± 39.93 and 207.87 ± 16.52 mg catechin equivalent/g dry weight of crude extract, respectively (Table 3).

Table 3. Total phenolic and flavonoid contents in crude Chandaeng extract.

Crude Chandaeng extract samples	Total phenolic contents (mg gallic/g dry weight of crude extract)	Total flavonoid contents (mg catechin/g dry weight of crude extract)
Methanol	356.05±1.17^{b}	492.34±39.93^{b}
Ethanol	357.35±0.85^{b}	667.39±40.11^{c}
DI	120.31±0.24^{a}	207.87±16.52^{a}

Mean values in the same column followed by a different letter were significantly different according to SPSS 16.0 by One-Way ANOVA (Tukey's test) at $p < 0.05$

Determination of loureirin B

HPLC chromatography revealed a peak of loureirin B in the extracts (Figure 3A), and the ethanolic extract (Figure 3B) showed a retention time for loureirin B of 10.614 min. The result of the analysis of loureirin B content in the ethanolic extract was calculated based on the standard (y = 26488.3272x - 1.5798, R2 = 0.9996) and showed 1.8% w/w (mg/g).

4. Discussion

In Asian nations, traditional plant-based remedies are frequently used to treat skin and tissue infections, and fever conditions, as well as for pain relief and anti-inflammatory applications, especially in China and Thailand. In this study, Chandaeng, a medicinal plant used in ancient remedies widely used in Thai households to treat infection-related ailments, was selected to undergo biological assays (Chusri et al. 2014). Pharmacological studies have found that the resin extracted from the stem of *D. cochinchinensis* has antibacterial, anti-inflammatory, analgesic, and anti-tumor activities (Chen et al. 1999; Xiang et al. 2001; He et al. 2006). According to a previous report, *Dracaena* produces phenolic compounds for plant defense following a fungal infection, and these compounds have the capability to be used in anti-bacterial and anti-

inflammation applications. Dragon's Blood resin from *D. cochinchinensis* was tested for antibacterial effects in mice against *S. aureus*, *S. lemon,* and *S. diphtheria* with a MIC value of 3.12 mg/kg (Chen et al. 1999). *D. cinnabari* Balf f. crude extract also inhibited *S. aureus*, *S. saprophyticus* and *E. coli* (Gupta et al. 2013; Altwair et al. 2015).

This study revealed that crude methanolic and ethanolic Chandaeng extracts could inhibit skin pathogenic bacteria including *S. aureus*, *S. epidermidis*, and *C. acnes* with MIC and MBC values ranging from 3.125–6.25 mg/mL. It has been reported that crude Chandaeng extract contains loureirin A, loureirin B, loureirin C, cochinchinenin, ethyl parabens, and 7,4-dihydroxyflavan and 7-hydroxy-4-methoxyflavan, with functions in antifungal, antibacterial, and anti-inflammatory activity (Fan et al. 2014). In the present study, after bacterial cells were treated with the crude Chandaeng extract for 2 h, they were seen to be a conglomerate, exhibiting collapse and cell lysis. Based on the results of a time-kill assay and scanning electron microscopy observations, the crude ethanolic extract of Chandaeng disrupted all of the tested microbial cells. Generally, the outer membranes of Gram-negative bacteria behave as a protective barrier, limiting the rate of penetration for some antimicrobial agents and rejecting bigger molecules, and allowing only tiny hydrophilic molecules to get through inside the cell. They also have multi-drug resistance pumps that inhibit some antibacterial molecules from passing the membrane (Lambert, 2002). The findings also indicated that the extract's effects on bacterial growth inhibition increased with extract concentration. After treatment, some cells shrink in order to reduce the target location for anti-microbial molecule attachment to the cell, hence not all shrunken cells actually reflect cell death. (Supardy et al. 2012). Cells are finally destroyed by a prolonged treatment period and an increase in the quantity of plant extract (Witkowska et al. 2013).

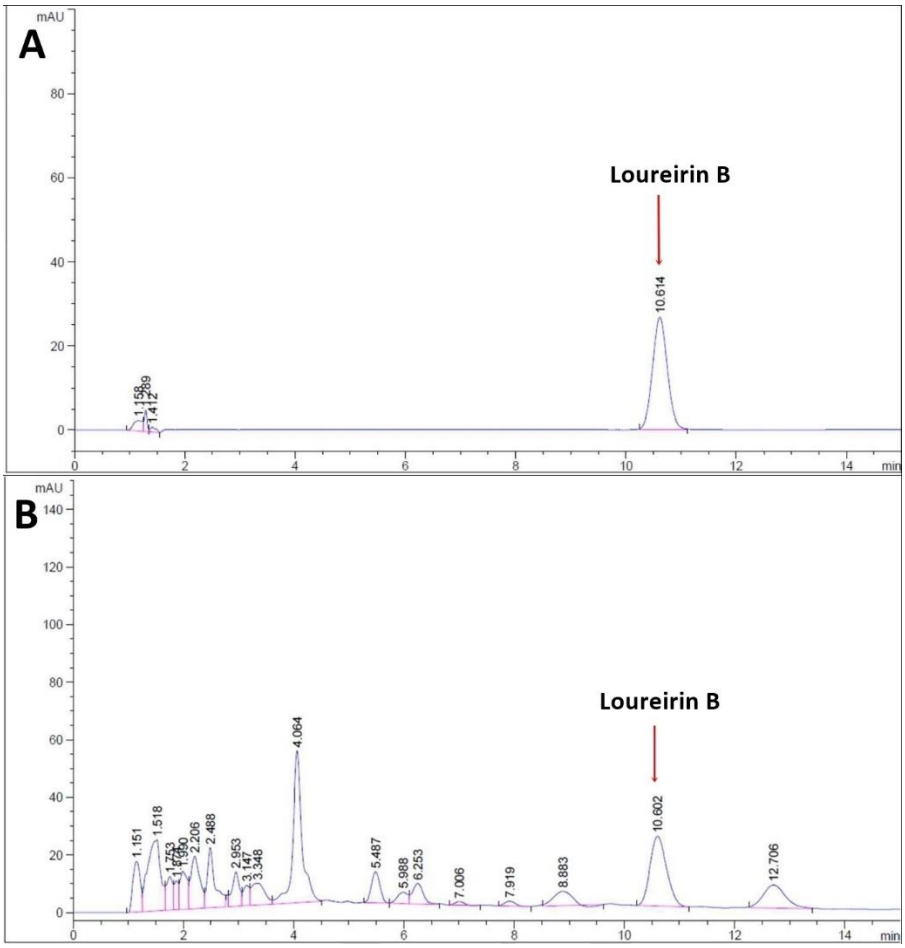

Figure 3. HPLC chromatograph of loureirin B standard (A) and loureirin B in crude Chandaeng extract (B).

The finding that crude Chandaeng extracts have tyrosinase inhibiting effects is of great interest. The ethanolic extract showed the highest enzyme inhibition potential, with an IC_{50} at 98.11 ± 10.03 µg/mL. The hydroxyl groups of the phenolic compounds in the extracts might play a role in the process of tyrosinase inhibition by forming hydrogen bonds at the active site of the enzyme and inhibiting its activity. Several

tyrosinase inhibitors function by attaching hydroxyl groups to the active site of the enzyme, which causes steric hindrance or an alteration in conformation (Baek et al. 2008). Furthermore, antioxidant activity may be one of the key mechanisms underlying tyrosinase inhibitory action (Alam et al. 2011). The tyrosinase inhibitory activity of several medicinal plant extracts has been reported. For example, extracts from the fruiting bodies of *Pleurotus citrinopileatus* (2.80 ± 2.44% enzyme inhibition at 100 µg/mL) (Meng et al. 2011), *Myristga fragran* Houtt extract (80.6% enzyme inhibition at 2.5 mg/mL) (Paneerchelvan et al. 2015), *Cassia fistula* flower butanol extract (40% enzyme inhibition at 100 µg/mL) (Limtrakul et al. 2016), and *Zingiber cassumunar* Robx. crude rhizome extract (35.43 ± 0.47 at 1.67 mg/mL) (Sungthong et al. 2015) all inhibit tyrosinase activity. The inhibition of tyrosinase has been a long-term target in the cosmetics industry for skin whitening purposes. Therefore, crude Chandaeng extracts could be a promising natural source of tyrosinase inhibitors. Moreover, a clinical trial suggested that the healing agent Dragon's Blood is active, accessible, inexpensive, and safe (Jiang et al. 2017). The long-term toxicity of Guangxi Dragon's Blood in rabbits and mice was tested by feeding for 30 days, and the results showed no significant toxic reactions in any animals.

The crude Chandaeng extracts's free radical scavenging capabilities were evaluated utilizing DPPH and ABTS-based techniques. These methods have been used widely to determine the antioxidant capacities of plant extracts. The ethanolic extract exhibited higher antioxidant activity than the methanolic and DI extracts. Previously, (7E)-2,4-dihydroxy-1-methylstilbene, obtained through the methanolic extraction of *D*. *cochinchinensis*, showed high antioxidant activity using the DPPH and ABTS methods (Niu et al. 2020). Moreover, Gupta and Gupta (Gupta et al. 2013) reported that the crude methanolic extract of *D*. *cinnabari* Balf f. showed IC_{50} values of 0.0942 ± 0.0024 and 0.0041 ± 0.0002 mg/mL using the DPPH and ABTS methods, respectively. The ferric reducing antioxidant power (FRAP) method showed an IC_{50} at 1.366 ± 0.017 mg Trolox equivalents/g of crude extract. The antioxidant activity of ethanolic *D*. *Loureiri* Gagnep. extract was assessed using the DPPH method. It showed a similar IC_{50} value at 98.67 ± 1.52 µg/mL, and the FRAP method revealed an IC_{50} at 1,235.11 ± 33.35 mg GAE/100 g of crude extract (Sakunpak et al. 2012). Significant correlations were also found between the DPPH and ABTS results. The Folin-Ciocalteu technique was employed to calculate the total phenolic compounds. The total phenolic compound components of crude Chandaeng extracts found in this investigation were consistent with those reported in previous research on related plant species, including *D*. *cinnabari* Balf f., performed using the Folin-Ciocalteu method (177.358 ± 6.178 mg GAE/g extract) (Gupta et al. 2013), and *D*. *loureiri* Gagnep. (3,896.16 ± 13.41 mg GAE/100 g extract) (Sakunpak et al. 2012). These results indicate a relationship between phenolic compound concentration and their free radical scavenging activity and ferric reducing capacities in crude Chandaeng extracts. Therefore, the crude ethanolic Chandaeng extracts were found to be useful sources of antioxidant activity, which correlated with their levels of total phenolic compounds.

The chemical compositions of the crude extracts were tested by preliminary phytochemical screening. Terpenoids, flavonoids, phenolic compounds, saponins, and tannins were found in the crude extracts. A previous study reported that *D*. *Loureiri* Gagnep. extract contains several phytochemicals, including flavonoids, as the main components, along with terpenoids, saponins, lignin, phenolic compounds, and tannins (Sakunpak et al. 2012; Fan et al. 2014). Determination of loureirin B in the crude ethanolic Chandaeng extract revealed a value of 1.8% w/w. A sample of Dragon's Blood must have more than 0.4% w/w of loureirin B in order to meet the Chinese national quality standard WS3-082(Z-016)-99(Z) (Fan et al. 2014; Wang et al. 2011). Loureirin B has shown promise in the treatment of cardiovascular illnesses such as thrombosis and fibrosis, as well as in the inhibition of amyloid fibril production and stimulation of neuron regeneration (Jiang et al. 2017; Ospondpant et al. 2022). In addition, aromatic rings in compounds such as loureirin A, loureirin B, loureirin C, cochinchinenin, socotrin-4'-ol, 4',7-dihydroxyflavan, 4-methylcholest-7-ene-3-ol, ethylparaben, resveratrol, and hydroxyphenol, etc. have been found in the resin extract of Chandaeng (Fan et al. 2014). As a result, phenolic and flavonoid components are abundant in crude Chandaeng extracts. The significant antioxidant capabilities of phenolic compounds are widely recognized.

5. Conclusions

Crude Chandaeng extracts showed potential antibacterial activities against *C. acnes* DMST14916, *S. epidermidis* TISTR518, and *S. aureus* TISTR321. They contained high levels of total phenolics and flavonoids that correlated with high antioxidant capacity and tyrosinase inhibition effects. Therefore, these biological properties of crude Chandaeng extracts revealed that, in the future, this could be an important ingredient for skin care and cosmetic products with anti-acne and anti-oxidation activities.

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