© 2021 Journal of Pharmacy & Pharmacognosy Research, 9 (6), 813-823, 2021
ISSN 0719-4250
http://jppres.com/jppres

Original Article

Time-kill kinetics and mechanism of action of *Caesalpinia sappan* L. and *Ochna integerrima* (Lour.) Merr. water extracts against pathogenic bacteria

[Cinética de tiempo de muerte y mecanismo de acción de extractos acuosos de *Caesalpinia sappan* L. y *Ochna integerrima* (Lour.) Merr. contra bacterias patógenas]

Prapairat Seephonkai[1*], Sutthira Sedlak[2], Komgrit Wongpakam[2], Kusavadee Sangdee[3], Aphidech Sangdee[3]

[1]Nanotechnology Research Unit, Department of Chemistry, Faculty of Science, Mahasarakham University, Khamriang, Kantarawichai, Maha Sarakham, 44150, Thailand and Center of Excellence for Innovation in Chemistry (PERCH-CIC).
[2]Walairukhavej Botanical Research Institute, Mahasarakham University, Thailand.
[3]Microbiology Group, Biomedical Sciences Research Unit, Faculty of Medicine, Mahasarakham University, Thailand.
[4]Microbiology and Applied Microbiology Research Unit, Department of Biology, Faculty of Science, Mahasarakham University, Thailand.
*E-mail: prapairat.s@msu.ac.th

Abstract

Context: *Caesalpinia sappan* and *Ochna integerrima* are two native plants of Thailand and distributed widely throughout the country. Recently, water extracts from the stem bark of *C. sappan* and *O. integerrima* were found to exhibit potently antibacterial property but the antibacterial mechanism of action toward target bacteria has not been investigated yet.

Aims: To study time-kill kinetic and the mechanism of action of *C. sappan* and *O. integerrima* water extracts toward target bacteria.

Methods: The time-kill kinetic study of bacteriostatic or bactericidal activity against Gram-positive and Gram-negative pathogenic bacteria within 24 h experiment was conducted and the mechanism of action on cell morphology of the target bacteria by using scanning electron microscopy (SEM) were investigated.

Results: Both of the *C. sappan* and *O. integerrima* water extracts possessed bacteriostatic or bactericidal activity toward six tested pathogenic bacteria. The bacteriostatic or bactericidal activity depending on bacterial strains and the test concentrations (0.5, 1 and 2 × MIC) of these two extracts to the tested bacteria were observed. The bactericidal activity against all of the tested bacterial was evinced at 2 × MIC. The morphological alterations of the target bacterial cells of changing the cell size (decreased and increased), cell lysis and having cell cavity were detected by SEM after the treatment with *C. sappan* and *O. integerrima* water extracts.

Conclusions: The water extracts from the stem bark of *C. sappan* and *O. integerrima* exhibited bactericidal activity by changing the cell size, cell lysis and cell cavity of the tested bacteria cell morphology.

Keywords: antibacterial activity; *Caesalpinia sappan*; mechanism of action; *Ochna integerrima*; time-kill kinetic.

Resumen

Contexto: *Caesalpinia sappan* y *Ochna integerrima* son dos plantas nativas de Tailandia y se distribuyen ampliamente por todo el país. Recientemente, se descubrió que los extractos de agua de la corteza del tallo de *C. sappan* y *O. integerrima* exhiben propiedades antibacterianas potentes, pero aún no se ha investigado el mecanismo de acción antibacteriano hacia las bacterias diana.

Objetivos: Estudiar la cinética de tiempo de muerte y el mecanismo de acción de los extractos de agua de *C. sappan* y *O. integerrima* sobre las bacterias objetivo.

Métodos: Se realizó el estudio cinético de tiempo de muerte de la actividad bacteriostática o bactericida contra bacterias patógenas Gram-positivas y Gram-negativas dentro de las 24 horas del experimento y se investigó el mecanismo de acción sobre la morfología celular de las bacterias diana mediante el uso de microscopio electrónico de barrido (SEM).

Resultados: Los extractos acuosos de *C. sappan* y *O. integerrima* exhibieron actividad bacteriostática o bactericida hacia seis bacterias patógenas probadas. Se observó la actividad bacteriostática o bactericida dependiendo de las cepas bacterianas y las concentraciones de prueba (0,5; 1 y 2 × MIC) de estos dos extractos en las bacterias probadas, mientras que la actividad bactericida contra todas las bacterias probadas se evidenció a 2 × MIC. Las alteraciones morfológicas de las células bacterianas diana de cambio de tamaño celular (disminución y aumento), lisis celular y cavidad celular fueron detectadas por SEM después del tratamiento con extractos de agua de *C. sappan* y *O. integerrima*.

Conclusiones: Los extractos acuosos de la corteza del tallo de *C. sappan* y *O. integerrima* exhibieron actividad bactericida al cambiar el tamaño celular, la lisis celular y la cavidad celular de la morfología celular de las bacterias probadas.

Palabras Clave: actividad antibacteriana; *Caesalpinia sappan;* cinética de tiempo de muerte; mecanismo de acción; *Ochna integerrima*.

ARTICLE INFO
Received: May 3, 2021.
Received in revised form: June 11, 2021.
Accepted: June 12, 2021.
Available Online: June 17, 2021.

INTRODUCTION

Phenolic compounds are groups of phytochemical secondary metabolites. It contains numerous groups of compounds such as phenols, phenolic acids, or polyphenols (simple flavonoids, flavones, flavonols, tannins, coumarins, quinones, complex flavonoids and colored anthocyanins). These compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Das et al., 2010; Babbar et al., 2014). They have been studied for their potential properties and many of them exhibited antibacterial activity (Bouarab-Chibane et al., 2019a; 2019b). The antimicrobial capacity of polyphenols and flavonoids from plants has been investigated against a wide range of microorganisms (Ahn et al., 1991; Diker et al., 1991; Sakanaka et al., 1992; Borris, 1996; Cushnie and Lamb, 2005; Cŏté et al. 2010). Therefore, plant products or molecules are considered to be good natural antimicrobial or antibacterial sources that can be used to treat microbial or bacterial infections (Pawar, 2008; Fernebro, 2011; Srivastava et al., 2013).

Caesalpinia sappan (L.) (*Leguminosae*) is distributed widely in Southeast Asia and is commonly known as 'sappan wood' or 'Brazil wood'. It is a small thorny tree of 6-9 m height and 15-25 cm in diameter with a few prickly branches. Its wood is orange-red, hard, very heavy and straight-grained with a fine texture (Pawar, 2008). The heartwood of this plant has been traditionally used as a natural coloring agent in beverage, food, cosmetic and garment for long history (Badami et al. 2004; Wetwitayaklung et al., 2005; Siva, 2007; Karapanagiotis et al., 2008; Wu Toegel et al., 2012; Nirmal and Panichayupakaranant, 2015). In folk medicine, the heartwood of *C. sappan* is used for variety of medicinal purposes, for examples analgesic, thrombosis and tumor treatments (Soka, 1985), to activate blood circulation and remove stasis, and to use as an emmenagogue, analgesic or anti-inflammatory agent in China (Xie et al., 2000; The Chinese Pharmacopoeia Commission, 2005), an ingredient in vitiated conditions of *Pitta* (burning sensation, wounds, ulcers, leprosy, skin diseases, diarrhea, dysentery, epilepsy, menorrhagia, leucorrhoea, diabetes) according to Ayurveda of India (Kirtikar and Basu, 1987; Warrier et al., 1993), to cure rheumatism and inflammatory diseases and use as an emmenagogue, and homeostatic agents in Vietnam (Do, 2001), and to treat tuberculosis, diarrhea, dysentery, skin infections and anemia in Thailand (Sireeratawong et al., 2010).

Ochna integerrima (Lour.) Merr. (*Ochnaceae*) belongs to the genus *Ochna* with more than 80 species distributed in tropical Asia, Africa and America (Rendle, 1952). There are only two species of the genus *Ochna* which are *O. integerrima* and *O. kirkii* Oliv. found in Thailand (Smitinand and Larsen, 1970; Smitinand, 2001) and the species *O. integerrima* distributed widely in Thailand (Smitinand and Larsen, 1970). It is a small tree with average height over 1 m for five year old tree and grown as an ornamental garden plant due to its typically beautiful flower (Trang et al., 2018). In Vietnam, *O. integerrima* has been ranked as the rare and endangered species due to its high demand trade of the beautiful species (Trang et al., 2018). In Thai folk medicine the bark of this plant has been used as a digestive tonic and the root as an anthelmintic whereas in Indonesia an infusion of the root and leaf is used as an antidysenteric and an antipyretic (Perry, 1980). There is none of the record of traditional use of *O. integerrima* to treat bacterial infection or related symptoms.

In our continue search on antibacterial activity of local medicinal plants, the water extracts from the stem barks of *C. sappan* and *O. integerrima* was recently found to exhibit potent antibacterial properties against three Gram-positive; *Staphylococcus aureus* (MSSA) DMST 2933, *Staphylococcus aureus* (MRSA) DMST 20651 and *Bacillus cereus* ATCC11778, and three Gram-negative; *Escherichia coli* ATCC 25922, *Salmonella enterica* serovar Typhi DMST 22842 and *Shigella fleneri* DMST 4423, foodborne pathogenic bacteria (Seephonkai et al., 2021). It indicates that stem barks of these two plant extracts can be potentially used for the treatment of microbial infection or related applications. However, details on antibacterial mecha-

nisms of these two plant extracts on their target bacteria have not been investigated yet. Therefore, the aim of our study was to investigate antibacterial activity in terms of time-kill kinetic and mode of action of the *C. sappan* and *O. integerrima* water extracts toward six strains of the foodborne target's pathogenic bacteria.

MATERIAL AND METHODS

Chemicals and reagents

Tetracycline (Sigma, USA), Mueller Hinton Agar (Difco, USA), Mueller Hinton Broth (Difco, USA), glutaraldehyde (Sigma-Aldrich, Germany) and sucrose (Ajax FineChem Laboratory Chemicals, New Zealand) were used for the experiments.

Plant material and extraction

The stem barks of *C. sappan* (L.) and *O. integerrima* (Lour.) Merr. were collected from Maha Sarakham province, northeastern Thailand in May, 2018 (15°41′52″N, 103°13′36″E). The plant samples were collected by K. Wongpakam and identified by S. Sedlak, Walai Rukhavej Botanical Research Institute (WRBRI), Mahasarakham University (MSU) where the voucher specimens of Wongpakam 19-01 for *C. sappan* and Wongpakam 19-02 for *O. integerrima* were deposited. Dried plants (50 g) were extracted in distilled water (500 mL) by refluxing for 24 h. An aqueous solution was collected after filtration, and 100 mL of the aqueous solution was subjected to freeze dry (Heto Power Dry PL3000, Thermo Fisher Scientific, USA) to obtain the water extracts from the stem barks of *C. sappan* and *O. integerrima*.

Microbial strains and cultivation

Six pathogenic bacteria targets; three Gram-positive bacteria, *Staphylococcus aureus* (MSSA) DMST 2933, *Staphylococcus aureus* (MRSA) DMST 20651 and *Bacillus cereus* ATCC1178, and three Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Salmonella enterica* serovar Typhi DMST 22842 and *Shigella fleneri* DMST 4423, were sourced from Medical Microorganisms Department of Medical Sciences Thailand. The selected strains were cultured on Mueller Hinton Agar (MHA) at 37°C for 16–18 h. Then, single colony of bacteria pathogens was inoculated into Mueller Hinton Broth (MHB) at 37°C for 4 h with shaking at 250 rpm. After that, bacteria culture was adjusted to 4–5 × 10^6 CFU/mL and stored at 4°C before use for tested experiments.

Time-kill assay

The experiment of time-kill was done as described in the literatures (White et al., 1996; Aiyegoro et al., 2009). The concentrations of 0.5, 1 and 2 × MIC of the extracts was used to test against target bacteria along with the test tube of MHB without extract samples and MHB with tetracycline (250 µg/mL), a standard antibiotic compound, in the assay. After incubation (37°C), bacterial suspension in a small volume was taken at 0, 2, 4, and 24 h to dilute and spread onto the MHA plate in order to count the viable colony number. The experiment was done in three replicates and mean was calculated using Microsoft® Excel® 2016 (Microsoft Corporation, Redmond, USA). A graph between viable bacteria number (mean value) and time taken was plotted to evaluate the killing rate. Results with bacterial count reduction of 3 log 10 were considered to have bactericidal activity while the bacterial count reduction less than 3 log 10 were defined as bacteriostatic activity.

Scanning electron microscope experiment

The mechanism of action of the extracts on target bacterial cell morphology was investigated using scanning electron microscope (SEM) experiment as described in literature (Sangdee et al., 2018). The pathogen cells were treated with the extracts at 2 × MIC level of the concentration, and were then incubated (37°C, 16–18 h). Next, the bacterial cells were harvested and washed, and fixed with 2.5% glutaraldehyde in 5% sucrose. After that, the cell pellets were dehydrated and then applied to a membrane. Gold was used to sputter-coat onto a dried extract powder surface for preparing samples which were taken to morphologically examine under a SEM (JOEL Tsm-700 FSHL, Japan). The cell alterations caused by the

extract treatment were compared with those caused by tetracycline (250 µg/mL) and the control treatments.

Statistical analysis

The data from three replicates were presented as mean using Microsoft® Excel® 2016 (Microsoft Corporation, Redmond, USA). Then, the time-kill kinetics were plot using Microsoft® Excel® 2016.

RESULTS

Time-kill assay

The time-kill assay of *C. sappan* water extract was shown in Fig. 1. The *C. sappan* extract at the concentration of 0.5 × MIC had the bactericidal activity against *S. aureus* (MRSA) DMST 20651 (Fig. 1B), and bacteriostatic activity against *S. aureus* (MSSA) DMST 2933 (Fig. 1A) and *B. cereus* ATCC 11778 (Fig. 1C). This extract did not show antibacterial property against *E. coli* ATCC 25922, *S. flexneri* DMST 4423 and *S.* Typhi DMST 22842 (Fig. 1D-F) at same level of the concentration. When increasing the concentration of the extract up to 1 x MIC, the bactericidal activity against all of the tested Gram-positive bacterial strains (Fig. 1A-C), and the bacteriostatic property against two strains of Gram-negative bacterial pathogen *E. coli* ATCC 25922 (Fig. 1D) and *S. flexneri* DMST 4423 (Fig. 1E) were observed by 24 h. However, there was no activity of the extract on *S.* Typhi DMST 22842 (Fig. 1F). Also, increasing the concentration of the extract up to 2 × MIC resulted in bactericidal ability toward *S. aureus* (MSSA) DMST 2933, *S. aureus* (MRSA) DMST 20651, *B. cereus* ATCC 11778, *E. coli* ATCC 25922 by 24 h (Fig. 1A-D), whereas the bacteriostatic activity against the remaining bacterial strains was not cleared (Fig. 1E and 1F). These results suggested that the *C. sappan* extract had bacteriostatic or bactericidal activity at the tested concentration of 0.5-2 × MIC against the tested bacterial strains.

For the *O. integerrima* water extract, at 0.5 × MIC concentration, the bactericidal or bacteriostatic potency against *B. cereus* ATCC 11778 (Fig. 2C) and *S.* Typhi DMST 22842 (Fig. 2F) was determined by 24 h, respectively. No activity of the extract against the remaining strains was found. At higher concentration of 1 × MIC, the bacteriostatic activity against both stains of Gram-positive *Staphylococci* were observed by 24 h, but it did not show antibacterial effect toward *S. flexneri* DMST 4423. Complete eliminations of bactericidal activity of three tested strains including *B. cereus* ATCC 11778, *E. coli* ATCC 25922 and *S.* Typhi DMST 22842 (Fig. 2C, D, F) were achieved by 24 h treatments with 1 × MIC the extract concentration. Increasing the concentration up to 2 × MIC resulted in bactericidal activity against all bacterial strains by 24 h (Fig. 2A-F). These results indicated the bacteriostatic or bactericidal activity of the *O. integerrima* extract is confirmed within 24 h experiment.

The time-kill study of the Gram-negative *S. flexneri* DMST 4423 when treated with the *C. sappan* and *O. integerrima* extracts indicated inactive bacteriostatic and bactericidal activity results at the concentrations of 0.5 × MIC and 1 × MIC. The bactericidal effect was only observed at the highest tested concentration of 2 x MIC which were 1.56 and 6.24 mg/mL against *C. sappan* and *O. integerrima*, respectively (Fig. 1E and 2E). Therefore, both of the extracts had not much impact toward *S. flexneri* DMST 4423.

Scanning electron microscope experiment

The water extracts of *C. sappan* and *O. integerrima* treated with five pathogenic bacteria were examined by SEM. The results showed similar alteration effects of the *C. sappan* and *O. integerrima* extracts on cell morphology of the tested bacteria. SEM micrographs of both *S. aureus* MSSA and MRSA strains after 24 h treatment with the extracts revealed decreased or increased in cell size (Fig. 3C-D and Fig. 4C-D). Cell shape of the *B. cereus* ATCC 11778 (Fig. 5C-D) was abnormal when compared with their original size, showing both cases of bacterial cells size increased or decreased. Moreover, some other cells appearing bacterial cell cavities were also observed. Cell shapes of the *E. coli* ATCC 25922 (Fig. 6C-D) and *S.* Typhi DMST 24822 (Fig. 7C-D) were induced several alterations causing cell size changing (increased or de-

creased), cell lysis and cell cavity. However, it is clear from the micrographs that the number of damaged cells greatly outnumbers those of normal cells. These results indicate that the active compound in the extracts had some effects on the cytoplasmic membrane and cell wall of the bacteria. These effects resulted in an alteration in cytoplasmic membrane permeability and led to cell wall leakage. Untreated control of all bacterial cells appeared undamaged (Fig. 3-7A) whilst the treatment of all bacterial strains with standard antibiotic tetracycline induced several alterations as similar as treated with both C. *sappan* and *O. integerrima* extracts (Fig. 3-7B).

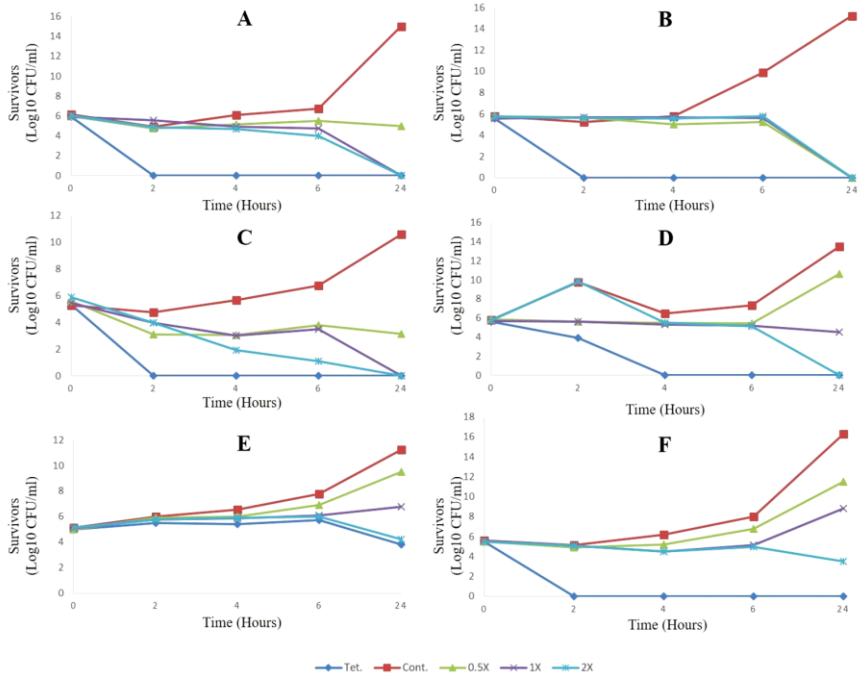

Figure 1. *In vitro* bacteriostatic and bacteriocidal activities of *C. sappan* water extract against *S. aureus* (MSSA) DMST 2933, MIC 0.78 mg/mL (**A**); *S. aureus* (MRSA) DMST 20651, MIC 0.78 mg/mL (**B**); *B. cereus* ATCC 11778 MIC 0.39 mg/mL (**C**); *E. coli* ATCC 25922, MIC 3.12 mg/mL (**D**); *S. flexneri* DMST 4423, MIC 0.78 mg/mL (**E**); and *S.* Typhi DMST 22842, MIC 0.78 mg/mL (**F**) at the concentration of 0.5 x MIC (▲), 1 x MIC (×), and 2 x MIC (∗) compared with the untreated control (■) and tetracycline at 250 µg/mL (♦). Data represent mean from three replicates.

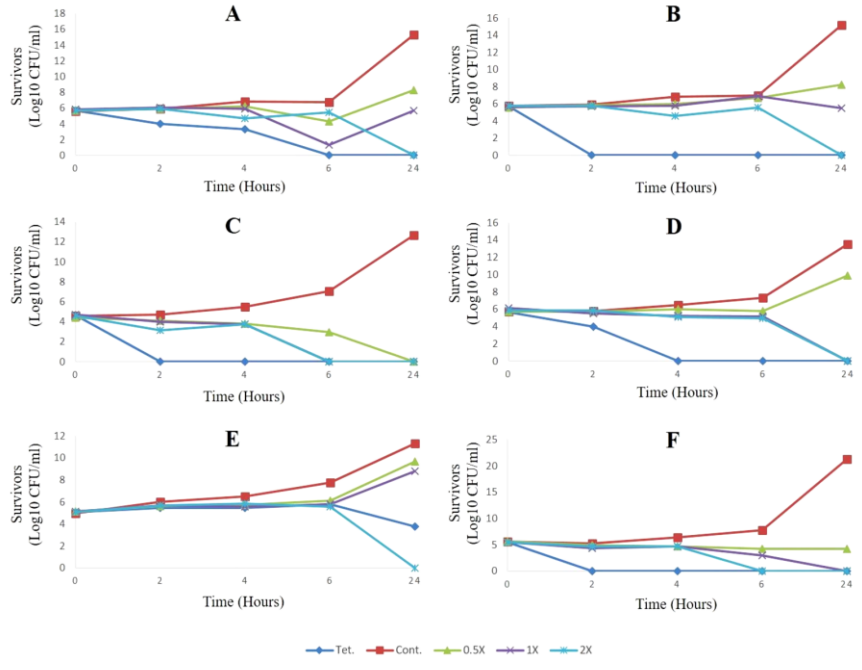

Figure 2. *In vitro* bacteriostatic and bacteriocidal activities of *O. integerrima* water extract against *S. aureus* (MSSA) DMST 2933, MIC 0.78 mg/mL (**A**); *S. aureus* (MRSA) DMST 20651, MIC 1.56 mg/mL (**B**); *B. cereus* ATCC 11778, MIC 0.78 mg/mL (**C**), *E. coli* ATCC 25922, MIC 6.25 mg/mL (**D**); *S. flexneri* DMST 4423, MIC 3.12 mg/mL (**E**); and *S.* Typhi DMST 22842, MIC 3.12 mg/mL (**F**) at the concentrations of 0.5 × MIC (▲), 1 × MIC (×) and 2 × MIC (∗) compared with the untreated control (■) and tetracycline at 250 µg/mL (♦). Data represent mean from three replicates.

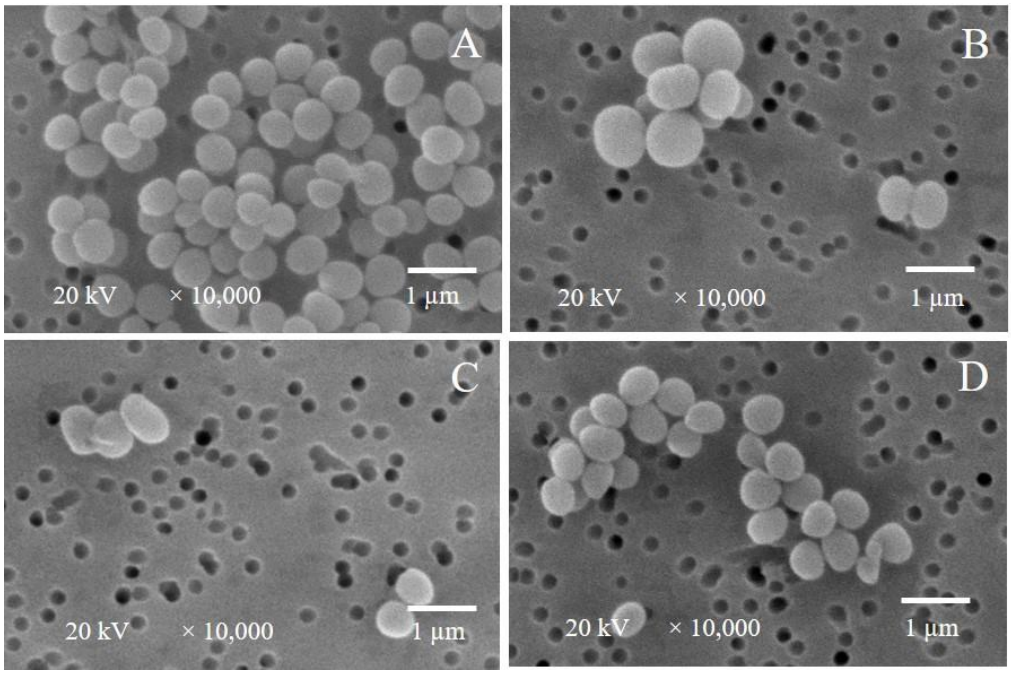

Figure 3. SEM micrographs of untreated control *S. aureus* (MSSA) DMST 2933 (**A**); compared with treated with tetracycline at 250 µg/mL (**B**); *C. sappan* water extract (**C**); and *O. integerrima* water extract (**D**).

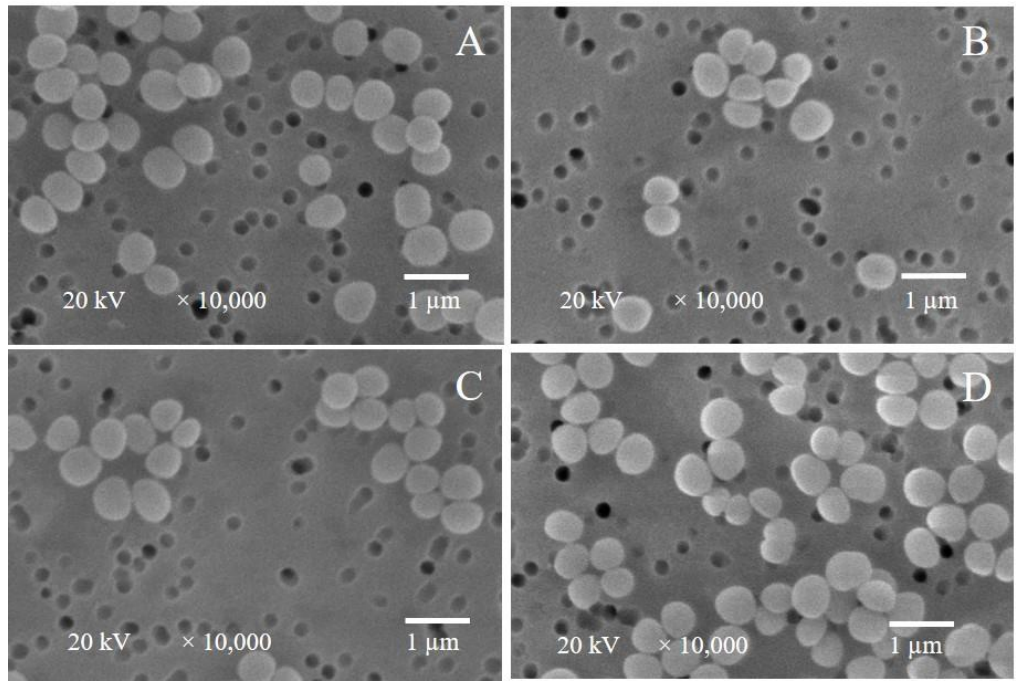

Figure 4. SEM micrographs of untreated control *S. aureus* (MRSA) DMST 20651 (**A**); compared with treated with tetracycline at 250 µg/mL (**B**); *C. sappan* water extract (**C**); and *O. integerrima* water extract (**D**).

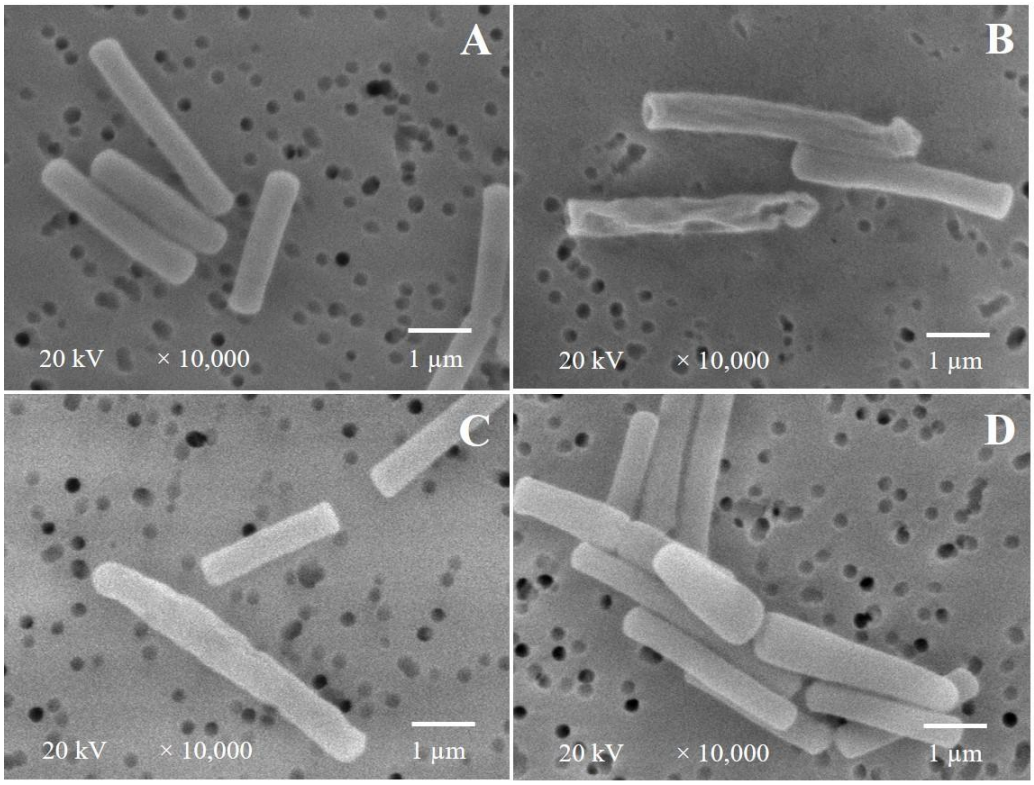

Figure 5. SEM micrographs of untreated control *B. cereus* ATCC 11778 (**A**); compared with treated with tetracycline at 250 µg/mL (**B**); *C. sappan* water extract (**C**); and *O. integerrima* water extract (**D**).

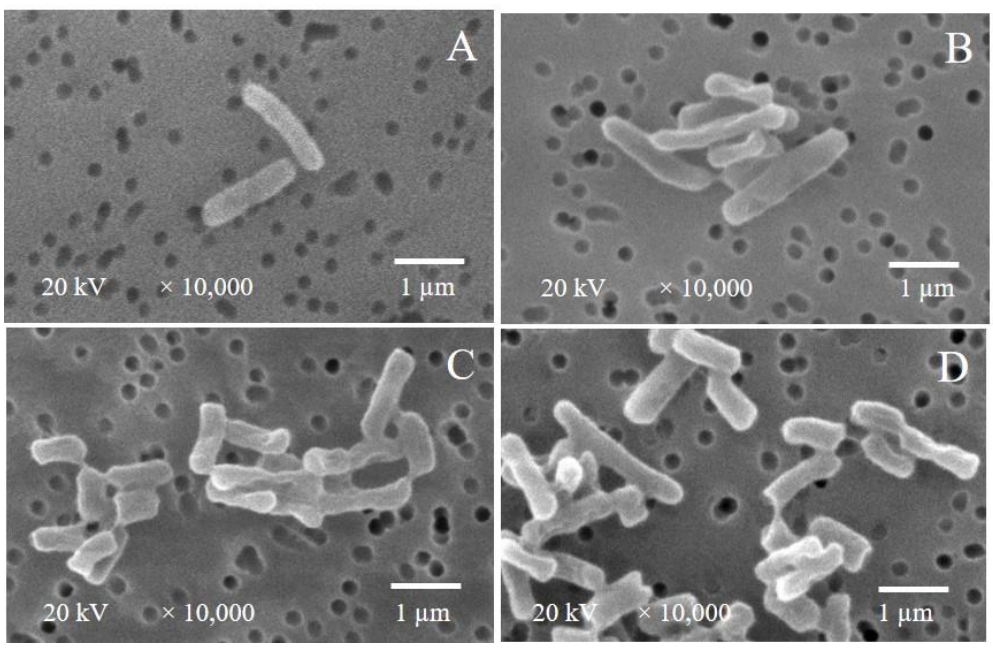

Figure 6. SEM micrographs of untreated control *E. coli* ATCC 25922 (**A**); compared with treated with tetracycline at 250 µg/mL (**B**); *C. sappan* water extract (**C**); and *O. integerrima* water extract (**D**).

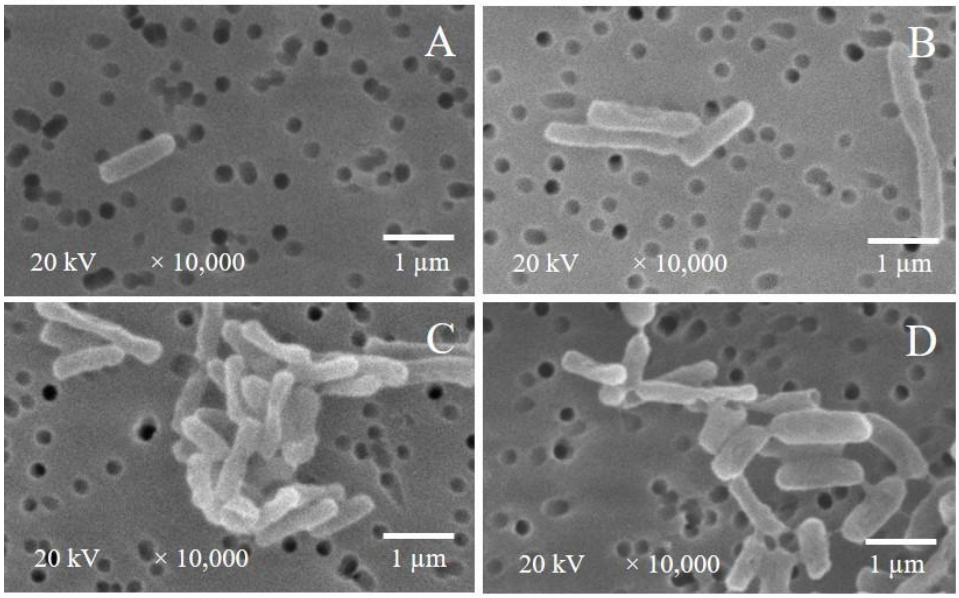

Figure 7. SEM micrographs of untreated control *S.* Typhi DMST 22842 (**A**); compared with treated with tetracycline at 250 µg/mL (**B**); *C. sappan* water extract (**C**); and *O. integerrima* water extract (**D**).

DISCUSSION

Nowadays, increasing in the incidence of drug-resistant bacterial pathogens infections have prompted renewed efforts to identify biologically active molecules from natural sources that could be useful for antibacterial therapies. Plant extracts have one of the candidate sources due to their varieties of bioactive phytochemicals and their safety for using on human health. According to our study, the microbial death kinetic and possible mechanism of action of the *C. sappan* and *O. integerrima* water extracts toward six foodborne pathogenic bacteria were first reported. The results demonstrated that the antibacterial activity depended on the extract and the strains of the tested bacteria. It indicates clearly that the *C. sappan* and *O. integerrima* extracts exhibited concentration- and time-dependent bacteriostatic or bactericidal activity.

Based on the results of death kinetic at the concentration of 2 × MIC level, the *C. sappan* extract exhibited greater antibacterial activity against the Gram-positive bacteria than Gram-negative bacteria. The extract had bactericidal property against all of the Gram-positive bacteria while the bacteriostatic activity was observed in *S. flexneri* DMST 4423 and *S.* Typhi DMST 22842. These results may be due to the mode of antibacterial action of the extract that responsible to the different target molecules in cell wall structure of the Gram-negative and Gram-positive bacteria (Russell, 2003). The *O. integerrima* extract at the same concentration of 2 × MIC possessed bactericidal ability against all of the tested bacterial strains by 24 h time-kill kinetic experiment. The antibacterial activity as bactericide of the *O. integerrima* extract seems to be stronger than the activity of *C. sappan* extract. The antibacterial activity of the *C. sappan* extract has been studies (Xu and Lee, 2004; Kim et al., 2004; Srinivasan et al., 2012; Nirmal and Panichayupakaranant, 2015) while the antibacterial activity of the *O. integerrima* extract has been explored by our group recently. However, the potent antibacterial property of *Ochna pretoriensis* species collected in South Africa against *E. coli* and *E. faecalis* has been documented (Makhafola and Eloff, 2012). Therefore, the stem bark of *O. integerrima* can be alternative potential plant sources of natural antibacterial substances according to our present results.

Results from the SEM images clearly showed bacterial cell morphological alterations such as cell lysis and cell cavity observing in the *B. cereus* ATCC 11778, *E. coli* ATCC 25922, *S. flexneri* DMST

4423 and *S.* Typhi DMST 22842 after the treatment with the *C. sappan* and *O. integerrima* extracts. These alterations may be due to flavonoid and phenolic compounds, which are groups of phytochemicals presented in the barks of *C. sappan* and *O. integerrima* extracts (Kaewamatawong et al., 2002; Ichino et al., 2006; Reutrakul et al., 2007; Pawar et al., 2008; Nirmal et al., 2015) that affecting the cell wall and cytoplasmic membrane of bacterial pathogens. Flavonoids represent another class of bioactive compounds that inhibit bacterial growth by involving the inhibition of DNA gyrase, disrupting the cytoplasmic membrane function and inhibiting of energy metabolism (Cushnie et al., 2016). Phenolic compounds also play an important role to inhibit bacterial cell growth by disrupting the cytoplasmic membrane of bacterial cell, then causing a change in membrane permeability and finally causing leakage of cytoplasmic constituents (Johnston et al., 2003). Therefore, the bacterial cell alteration changes found in this study may suggest that the mode of antibacterial action of the extract involves disruption of peptidoglycan biosynthesis (Cushnie et al., 2016).

CONCLUSIONS

Both of the *C. sappan* and *O. integerrima* extracts showed time- and concentration-dependent bacteriostatic or bactericidal activity against all of the tested pathogenic bacteria. The bacteriostatic or bactericidal property were observed at the tested concentrations of 0.5 × MIC and 2 × MIC while the bactericidal activity against all of the tested bacterial was found at 2 × MIC. The morphological alterations of changing the bacterial cell size, cell lysis and having cell cavity were observed.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

ACKNOWLEDGMENTS

This research was financially supported by Mahasarakham University (Grant year 2019). PS gratefuls the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Ministry of Higher Education, Research and Innovation and Faculty of Science, Mahasarakham University.

REFERENCES

Ahn YJ, Kawamura T, Kim M, Yamamoto T, Mitsuoka T (1991) Tea polyphenols: Selective growth inhibitors of *Clostridium* spp. Agric Biol Chem 551: 425–1426.

Aiyegoro OA, Afolayan AJ, Okoh AI (2009) *In vitro* antibacterial time kill studies of leaves extracts of *Helichrysum longifolium*. J Med Plant Res 3(6): 462–67.

Babbar N, Oberoi HS, Sandhu SK, Bhargav VK (2014) Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. J Food Sci Technol 51: 2568–2575.

Badami S, Moorkoth S, Suresh B (2004) *Caesalpinia sappan* a medicinal and dye yielding plant. Nat Prod Rad 3(2): 75–82.

Borris RP (1996) Natural products research: Perspectives from a major pharmaceutical company. J Ethnopharmacol 51: 29–38.

Bouarab-Chibane L, Degraeve P, Ferhout H, Bouajila J, Oulahal N (2019a) Plant antimicrobial polyphenols as potential natural food preservatives. J Sci Food Agric 99: 1457–1474.

Bouarab-Chibane L, Forquet V, Lantéri P, Clément Y, Léonard-Akkari L, Oulahal N, Degraeve P, Bordes C (2019b) Antibacterial properties of polyphenols: Characterization and QSAR (Quantitative Structure-Activity Relationship) models. Front Microbiol 10: 1–23.

Cŏté J, Caillet S, Doyon G, Sylvain JP, Lacroix M (2010) Bioactive compounds in cranberries and their biological properties. Crit Rev Food Sci Nutr 50: 666–679.

Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005; 26: 343–356.

Cushnie TPT, O'Driscoll NH, Lamb AJ (2016) Morphological and ultrastructural changes in bacterial cells as an indicator of antibacterial mechanism of action. Cell Mol Life Sci 73: 4471–92.

Das K, Tiwari RKS, Shrivastava DK (2010) Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plant Res 4(2): 104–111.

Diker KS, Akan M, Hascelik G, Yurdakök M (1991) The bacterial activity of tea against *Campylobacter jejuni* and *Campylobacter coli*. Lett App Microbiol 12: 34–35.

Do TL (2001) Vietnamese Medicinal Plants; Medicine Publisher: Hanoi, p. 50.

Fernebro J (2011) Fighting bacterial infections - Future treatment options. Drug Resist Updates 14: 125–39.

Ichino C, Kiyohara H, Soonthornchareonnon N, Chuakul W, Ishiyama A, Sekiguchi H, Namatame M, Otoguro K, Omura S, Yamada H (2006) Antimalarial activity of biflavonoids from *Ochna integerrima*. Planta Med 72(7): 611–614.

Johnston MD, Hanlon GW, Denyer SP, Lambert RJW (2003) Membrane damage to bacteria caused by single and combined biocides. J Appl Microbiol 94: 1015–1023.

Kaewamatawong R, Likhitwitayawuid K, Ruangrungsi N, Takayama H, Kitajima M, Aimi N (2002) Novel biflavonoids from the stem bark of *Ochna integerrima*. J Nat Prod 65: 1027–1029.

Karapanagiotis I, Lakka A, Valianou L, Chryssoulakis Y (2008) High-performance liquid chromatographic determination of colouring matters in historical garments from the Holy Mountain of Athos. MCA 160: 477–483.

Kim KJ, Yu HH, Jeong SI, Cha JD, Kim SM, You YO (2004) Inhibitory effects of *Caesalpinia sappan* on growth and invasion of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol 91(1): 81–87.

Kirtikar KR, Basu BD (1987) Indian Medicinal Plants. International Book Distributors, Dehradun, India, pp. 847–848.

Makhafola TJ, Eloff JN (2012) Five *Ochna* species have high antibacterial activity and more than ten antibacterial compounds. S Afr J Sci 108(1/2): 1–6.

Nirmal NP, Panichayupakaranant P (2015) Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich *Caesalpinia sappan* extract. Pharm Biol 53(9): 1339–1343.

Nirmal NP, Rajput MS, Prasad RGSV, Ahmad M (2015) Brazillin from *Caesalpinia sappan* heartwood and its pharmacological activities: A review. Asian Pac J Trop Med 8(6): 421–430.

Pawar CR, Landge AD, Surana SJ (2008) Phytochemical and pharmacological aspects of *Caesalpinia sappan*. J Pharm Res 1(2): 131–138.

Rendle AB (1952) The Classification of Flowering Plants vol. 2, Cambridge University Press London.

Perry LM (1980) Medicinal Plants of East and Southeast Asia, Boston, The MIT Press, pp. 289-290.

Reutrakul V, Ningnuek N, Pohmakotr M, Yoosook C, Napaswad C, Kasisit J, Santisuk T, Tuchinda P (2007) Anti-HIV-1 flavonoid glycosides from *Ochna integerrima*. Planta Med 73(7): 683v–688.

Russell AD (2003) Similarities and differences in the responses of microorganisms to biocides. J Antimicrob Chemother 52: 750–63.

Sakanaka S, Shimura N, Aizawa M, Kim M, Yamamoto T (1992) Preventive effect of green tea polyphenols against dental caries in conventional rats. Biosci Biotech Bioch 56: 592–594.

Sangdee A, Sangdee K, Buranrat B, Thammawat S (2018) Effects of mycelial extract and crude protein of the medicinal mushroom, *Ophiocordyceps sobolifera*, on the pathogenic fungus, *Candida albicans*. Trop J Pharm Res 17(12): 2449–2454.

Seephonkai P, Mongkolsiri N, Thiabphet W, Traisathit R, Sedlak S, Wongpakam K, Dhammaraj T, Sangdee A (2021) Antioxidant, xanthine oxidase inhibitory and antibacterial activities of selected galactogogue Thai medicinal plant water and ethyl acetate extracts. J Res Pharm (http://www.jrespharm.com/abstract.php?lang=en&id=908) [June 10, 2021].

Sireeratawong S, Piyabhan P, Singhalak T, Wongkrajang Y, Temsiririrkkul R, Punsrirat J, Ruangwises N, Saraya S, Lerdvuthisopon N, Jaijoy K (2010) Toxicity evaluation of sappan wood extract in rats. J Med Assoc Thai 93: S50–S57.

Siva R (2007) Status of natural dyes and dye-yielding plants in India. Curr Sci 92: 916–925.

Smitinand T, Larsen K (1970) Flora of Thailand, Tistr Press, Bangkok.

Smitinand T (2001) Thai Plant Names, Funny Publishing, Bangkok, Thailand.

Soka T (1985) Dictionary of Chinese Drugs, Shanghai Science Technology Shogaukan (Eds.), Shogakukan Press, Tokyo, pp. 1627–1628.

Srinivasan R, Selvam GG, Karthik S, Mathivanan K, Baskaran R, Karthikeyan M, Gopi M, Govindasamy C (2012) *In vitro* antimicrobial activity of *Caesalpinia sappan* L. Asian Pac J. Trop Biomed 2(1): 136–139.

Srivastava J, Chandra H, Nautiyal AR, Kalra SJS (2013) Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. 3 Biotech 4: 451–60.

The Chinese Pharmacopoeia Commission (2005) The Pharmacopoeia of the People's Republic of China, (Part I), Chemical Industry Press, Beijing, China, p. 113.

Trang DH, Dong DV, Chung BH, Gioi DH, Khanh TD (2018) Identification of Vietnamese *Ochna integerrima* (Lour.) Merr species based on ribosomal DNA internal transcribed spacer sequence. Int Lett Nat Sci 68: 9–18.

Warrier PK, Nambiar VPK, Ramankutty C (1993) Indian Medicinal Plants, A Compendium of 500 Species," Vol. 1, Orient Longman Ltd., Chennai, India, pp. 291–293.

Wetwitayaklung P, Phaechawud T, Keokitichai S (2005) Antioxidant activity of *Caesalpinia sappan* L. heartwood in various ages. NUJST 3(2): 43-–52.

White RL, Burgess DS, Manduru M, Bosso JA (1996) Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. Antimicrob Agents Chemother 40(8): 1914–18.

Wu Toegel SQ, Otero M, Goldring MB, Leelapornpisid P, Chiari C, Kolb A, Unger FM, Windhager R, Viernstein H (2012) *Caesalpinia sappan* extract inhibits IL1β-mediate overexpression of matrix metalloproteinase in human chondrocytes. Genes Nutr 7(2): 312–318.

Xie YW, Ming DS, Xu HX, Dong H, But PP (2000) Vasorelaxing effects of *Caesalpinia sappan* involvement of endogenous nitric oxide. Life Sci 67: 1913–1918.

Xu HX, Lee SF (2004) The antibacterial principle of *Caesalpinia sappan*. Phytother Res 18(8): 647–651.

AUTHOR CONTRIBUTION:

Contribution	Seephonkai P	Sedlak S	Wongpakam K	Sangdee K	Sangdee A
Concepts or ideas	x	x	x	x	x
Design	x	x	x	x	x
Definition of intellectual content	x	x	x		x
Literature search	x	x	x		x
Experimental studies	x	x	x	x	x
Data acquisition	x	x	x	x	x
Data analysis	x	x	x	x	x
Statistical analysis	x				x
Manuscript preparation	x	x	x		x
Manuscript editing	x	x	x	x	x
Manuscript review	x	x	x	x	x

Citation Format: Seephonkai P, Sedlak S, Wongpakam K, Sangdee K, Sangdee A (2021) Time-kill kinetics and mechanism of action of *Caesalpinia sappan* L. and *Ochna integerrima* (Lour.) Merr. water extracts against pathogenic bacteria. J Pharm Pharmacogn Res 9(6): 813–823.