P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia
Science & Technology Asia
Vol.25 No.3 July - September 2020
Page: [78-86]
Original research article

Antibacterial Activity of Mahajak Remedy and Plant Ingredients

Phadungnaree Saenmuang[1], Sumalee Panthong [1,2], Arunporn Intharat[1,2,*]

[1]*Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*
[2]*Center of Excellence in Applied Thai Traditional Medicine Research, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*

Received 28 October 2019; Received in revised form 20 June 2020
Accepted 26 June 2020; Available online 21 September 2020

ABSTRACT

Mahajak (MH) oil is a remedy in Tamra-Osodpranarai. It is used for relieving pain, and skin infection as a Thai traditional medicine. This study investigated the efficacy of the MH remedy and plant ingredient extract on antibacterial activities. The MH remedy was macerated with sesame oil, 95% ethanol, hexane, and its plant ingredients were macerated with 95% ethanol. Then, all extracts were concentrated by rotary evaporator. The extracts were investigated on antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, and *Bacillus subtilis* ATCC 6633 by disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results show that the 95% ethanol extract of MH remedy was the best extract of antibacterial testing. Using the disc containing 10 µL (concentrate 500 mg/mL of the extracts), the inhibition zone was observed against *S. aureus* (9.67±0.33 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 2.50 mg/mL were obtained for residue hexane. Hexane extracts showed MIC and MBC of *S.aureus* and *B.subtilis* with similar values to 95% ethanol. However, all of the tests had no inhibitory activity against *P.aeruginosa*. In conclusion, this study demonstrated that 95% ethanol extracts of the MH remedy have antibacterial activity and may be a potential treatment in cases of skin infection.

Keywords: Antibacterial activities; Mahajak oil; Tamra-osodpranarai

***Corresponding author:** iarunporn@yahoo.com **doi:** 10.14456/scitechasia.2020.37

1. Introduction

Bacterial skin infections are caused by many types of bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [1]. Skin infection includes cellulitis, erysipelas, impetigo, folliculitis, furuncles and carbuncles, which can be classified by the depth and skin compartment [2, 3]. The World Health Organization (WHO) has estimated that 56.9 million people died worldwide in 2016, and infectious diseases were one cause of death [4]. Antibiotics, such as penicillin, clindamycin, and cephalosporin, are widely used to treat skin infection [5]. However, bacteria that still survive on antibiotic treatment are called antibiotic-resistant. Antibiotic resistance has become a global health problem. In 2018, the National Antimicrobial Resistance Surveillance Center in Thailand reported that antibiotic resistance was found in all parts of Thailand, such as *S. aureus* resistant to methicillin [6]. At the present time, many researchers are interested in an antimicrobial agent from a natural product. Secondary metabolites from plants, such as tannins, alkaloids, and quinones, can inhibit pathogens. Thus, many natural products have been investigated as antimicrobial agents and in the development of antimicrobial agents [7]. In Thailand, traditional Thai doctors have used Thai traditional remedies that combine many types of herbal plant as a drug for the treatment of patients for a long time. Mahajak (MH) is a traditional Thai remedy which has been described in the Osodphranarai scripture. It has long been used to relieve pain and reduce skin inflammation. At present, the MH remedy is still used by Thai Traditional doctors in Lopburi's hospital to treat skin inflamemation. The MH remedy consists of *Nigella sativa* L., *Lepidium sativum* L., *Cuminum cyminum* L., *Foeniculum vulgare* Miller., *Anethum graveolens* L., *Piper retrofractum* Vahl., *Citrus hystrix* DC., and *Sesamum indicum* L. [8,9].

However, there is no scientific report on the antibacterial activity of this remedy. Thus, the objective of this study was to investigate the antibacterial activity of MH extracts involved in skin infection and inflammation.

2. Materials and methods

2.1 Plant materials

The MH remedy, including *Nigella sativa* L, *Lepidium sativum* L, *Cuminum cyminum* L, *Foeniculum vulgare* Miller, *Anethum graveolens* L., and *Piper retrofractum* Vahl., was obtained from a Thai herbal drug store in Bangkok. The ingredients were confirmed by comparison with authentic voucher specimens that were kept in the herbarium of Southern Center of Thai Medicinal Plants, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. The herbarium voucher specimen number is shown in (Table 1). Plant ingredients were washed with water to remove contamination and dried at 50ºC for 72 hr. Each plant was ground to powder and weighed according to the recipe of the MH remedy in the Osodpranarai scripture.

2.2 Preparation of crude extract

Plant ingredients of the MH remedy were extracted by the traditional method and conventional method. For the traditional method, plant ingredients were macerated in sesame oil, while an organic solvent such as ethanol and hexane were used for the conventional extraction method.

2.2.1 Maceration in sesame oil

Firstly, fresh peel of kaffir lime was added into sesame oil (the ratio of 2:1) and heated at 150ºC for 25-30 min. Then, the sesame oil was filtered, cooled down to room temperature, and used for maceration of plant ingredients. Plant powder ingredients were separated into two parts. The first part and second part of plant ingredients were macerated with a sesame oil volume of 600 mL and 200 mL for 15 days to obtain MH oil

remedy extract (MH1) and MH oil concentrate extract (MH2).

2.2.2 Maceration in an organic solvent

The crude powder was extracted by maceration with hexane and 95% ethanol for three days and filtered through Whatman filter paper no1. The maceration process was repeated twice more with hexane and 95% ethanol. The hexane extract (MH3) and ethanolic extract (MH4) were concentrated by rotary evaporator, vacuum dried for one day, and stored at -20ºC until used. The residues of MH1, MH2, and MH3 were extracted by maceration with 95% ethanol and used the same procedure as MH4 extraction. Finally, we obtained MH (Oil remedy) extract (MH5), residue of MH (Oil concentrate) extract (MH6), the residue of hexane extract (MH7).

The yields of MH1- MH7 extracts were calculated as the percentage of yield by using the following equation:

$$\%\text{Yield} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried crude power (g)}} \times 100$$

2.3 Quality control of plant ingredients [10]

All plant ingredients were tested for quality control including loss on drying, extractive value, total ash, and insoluble acid ash by using the guidelines of the Thai Herbal Pharmacopoeia.

2.3.1 Loss on drying

The electronic moisture analyzer was used for the analysis of loss on drying. Each plant (2 g) was put into the moisture analyzer and heated to 105°C. The weight (W) of the dried sample was taken and analyzed by using the following equation.

$$\%\text{Loss on drying} = \frac{\text{W start (g)} - \text{W dried}}{\text{W start (g)}} \times 100$$

2.3.2 Ethanol-soluble extractive value

Five grams of the powdered drug was macerated with 100 mL of ethanol in a flask that was closed with foil for 24 hrs. Moreover, it was occasionally shaken over the first 6 hrs. Then, it was allowed to stand for 18 hrs. and filtered. After filtration, 25 mL of the filtrate was evaporated at 105°C to dryness in a tare flat-bottomed shallow dish, then dried and weighed. Percentage of ethanol-soluble extractive value was calculated with the below formula

$$\%\text{Extractive value} = \frac{\text{W extract (g)}}{\text{W dried plant material (g)}} \times 100$$

2.3.3 Total ash

This method was used to investigate the physiological ash and non-physiological ash or inorganic compounds of plant material.

Table 1. Plant materials of MH remedy.

Scientific Name	Family Name	Thai Name	Part	Voucher specimen number	Weight formulation
Nigella sativa L.	Ranunculaceae	Thian-Dam	Seed	SKP 160 14 19 01	15 g
Lepidium sativum L.	Cruciferae	Thian-Daeng	Seed	SKP 057 12 19 01	15 g
Cuminum cyminum L.	Umbelliferae	Thian-Khao	Seed	SKP 199 03 03 01	15 g
Foeniculum vulgare Miller subsp.	Umbelliferae	Thian-Khaoplueak	Seed	SKP 199 06 22 01	15 g
Anethum graveolens L.	Umbelliferae	Thian-Tatakkataen	Seed	SKP 199 01 07 01	15 g
Piper retrofractum Vahl.	Piperaceae	Dipli	Fruit	SKP 146 16 18 01	30 g

Total ash was determined by weighing 2 g into a pre-weighed crucible and burning in a muffle furnace at 450°C for 5 hr. Then, the crucible was placed into the desiccator to cool down. Finally, crucible with ash was weighed again. The percentage of total ash was calculated by using the following equation.

$$\%\text{Total ash} = \frac{\text{W after burning (g)}}{\text{W before burning (g)}} \times 100$$

2.3.4 Acid insoluble ash

The ash obtained from the previous process was boiled with 25 mL of 10 % HCl for 5 min. The insoluble matter was collected on ash-less filter paper and washed with DI water (pH 7). Ash-less filter paper with the insoluble matter was dried and weight. After that, it was burned and cooled in a desiccator. Finally, it was weighed and the percentage of acid insoluble ash was calculated by using the following equation.

$$\%\text{Acid insoluble ash} = \frac{\text{W after burning (g)}}{\text{W before burning (g)}} \times 100$$

2.4 Anti-bacterial Assay

The microorganisms used in this study were obtained from the Faculty of Medicine, Thammasat University, Khlong Luang, Pathum Thani, Thailand. The reference bacterial strains were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, and *Bacillus subtilis* ATCC 6633. All of the bacteria were cultured on nutrient agar and incubated for 24 hr. at 37ºC before testing.

2.4.1 Antibacterial assay by Disc diffusion method [11]

The disc diffusion was used to screen the antibacterial activity of extracts. All extracts were prepared at 500 mg/mL in DMSO and transferred (10 µL) to paper disc. Each bacterium was inoculated in Mueller Hinton broth (MHB) and incubated at 37ºC for 2 hr. After that, it was adjusted to 0.5 McFarland by using a McFarland densitometer. Then, 0.5 McFarland of each bacterium was swabbed on Mueller Hinton agar (MHA). Air-dried discs were placed on the inoculated MHA surface. An antibiotic commercial disc of ampicillin 10 µg/disc was used as a positive control. Negative control was a disc that contained 10 µL of 2% DMSO. Plates were incubated at 37ºC for 18-24 hr. After incubation, the inhibition zone diameter was measured. These tests were performed in triplicate.

2.4.2 Determination of minimum inhibitory concentration (MIC) [12]

The MIC value was determined by a broth dilution method. Briefly, all extracts were dissolved in 2% dimethylsulfoxide (DMSO) in which the maximum final concentration was 5 mg/mL. The serial two-fold dilution was performed in a concentration range of 0.039-5.00 mg/mL in a 96-well plate (50 µL/well). Then, 50 µL of 0.5 McFarland of the tested microorganisms was added to each well and incubated at 37°C for 24 hr. After that, 10 µl of resazurin (5 mg/mL) was added into each well and incubated at 37°C for 2 hr. The final concentration that did not show a color change of resazurin from blue to pink was the MIC value.

2.4.3 Determination of minimum bactericidal concentration (MBC) [13]

The MBC value is the lowest extract concentration of extract which can kill bacteria at least 99.9% within 24 hr. In short, the contents in the MIC well and those above the MIC for each micro-organism was streaked on MHA and incubated at 37°C for 24 hr. After incubation, the bacteria colonies on MHA were observed. The final concentration that bacteria colony did not appear was the MBC value.

3. Results and Discussion

MH1 showed the highest yield with a percent yield value of 61.17%. Moreover, MH2 and MH7 showed a high percent yield with a percentage of yield of 40.68% and 35.67%, respectively, as shown in Table 2. TD1 showed the highest yield (32.80%)

when compared with other plant ingredients. Moreover, TK1 and DP also showed a high percent yield with 10.99% and 10.47%.

Quality control of plant ingredients of the Mahajak remedy was performed including extractive value testing, loss on drying testing, total ash testing, and acid insoluble ash testing, as shown in Table 3. The results of extractive value showed that TD2, TD1, and TK3 showed high extractive values of 23.69%, 14.67%, and 11.50%, respectively. For loss on drying, we found that all samples passed the standard criteria of the Thai Herbal Pharmacopeia.

The results are shown in quality control of the MH remedy and plant ingredients. To determine the contamination of sand, soil, and siliceous earth in plant ingredients, total ash and insoluble acid ash of plant were performed. All plant materials displayed the total ash value of percent total ash between 0.43-7.15% and the insoluble acid ash of percent acid insoluble ash between 0.62-1.76%.

The MH remedy extracts and its plant ingredients extracts were investigated for antibacterial activity by using the disk diffusion method. All extracts have no inhibitory effect on *P. aeruginosa*. MH4 showed the best activity to inhibit *S. aureus* and *B. subtilis* with an inhibition zone of 9.67 and 10.33 mm, respectively, as shown in Table 4.

Table 2. The percentage yield of MH extracts and its plant ingredients.

Sample	Code	% Yield
Mahajak (Oil remedy) extract	MH1	61.17
Mahajak (Oil concentrate) extract	MH2	40.68
Hexane extract	MH3	21.09
95% Ethanol extract	MH4	25.11
The residue of MH (Oil remedy)extract	MH5	27.77
Residue of MH (Oil concentrate) extract	MH6	20.43
Residue of Hexane extract	MH7	35.67
Nigella sativa L.	TD1	32.80
Lepidium sativum L.	TD2	8.01
Cuminum cyminum L.	TK1	10.99
Foeniculum vulgare Miller subsp.	TK2	8.29
Anethum graveolens L.	TK3	6.07
Piper retrofractum Vahl.	DP	10.47

Table 3. Quality control of MH remedy extract and plant ingredients.

Plant	% Extractive value	Loss on drying		% Total Ash	% Total Acid
		Criteria standard	% Loss on drying	Standard <10%	Standard <2%
Nigella sativa L.	14.67± 0.47	7 %	5.72 ± 0.37	0.43 ± 0.02	0.62 ± 0.19
Lepidium sativum L.	23.69 ± 0.36	10 %	6.72 ± 0.09	5.10 ± 0.02	0.91 ± 0.14
Cuminum cyminum L.	6.10 ± 0.32	10 %	9.21 ± 0.08	5.57 ± 0.02	1.44 ± 0.10
Foeniculum vulgare Miller subsp.	10.04 ± 0.25	10 %	7.52 ± 0.22	7.15± 0.11	1.34 ± 0.18
Anethum graveolens L.	11.50 ± 0.12	9 %	7.44 ± 0.28	5.48 ± 0.08	0.69 ± 0.12
Piper retrofractum Vahl.	3.56 ± 0.02	13 %	9.63 ± 0.28	6.45 ± 0.08	1.25 ± 0.10
MH remedy	6.50 ± 0.05	10 %	3.76 ± 0.37	6.04 ± 0.26	1.76 ± 0.07

Moreover, MH3 and MH7 showed antibacterial activity against *S. aureus* with an inhibition zone of 9.33 and 8.33 mm, respectively. However, some MH extract showed no effect against *S. aureus* and *B. subtilis* such as MH1, MH2, MH5, and MH6.

Table 4. Antibacterial activities of MH remedy extracts against bacterial test organisms.

MH extracts	Zone of inhibition in mm.		
	S. aureus	*P. aeruginosa*	*B. subtilis*
MH1	NI	NI	NI
MH2	NI	NI	NI
MH3	NI	NI	9.33±0.33
MH4	9.67±0.33	NI	10.33±0.67
MH5	NI	NI	NI
MH6	NI	NI	NI
MH7	NI	NI	8.33±0.33
AM	28.33±0.17	NI	30.13±0.09

All extracts were tested for antibacterial activity by using a microtiter plate-based method to confirm their antibacterial activity. The MH4 extract showed the best antibacterial activity against *S. aureus* and *B. subtilis* with MIC values of 2.50 and 1.25 mg/mL and MBC values of 2.50 and 2.50 mg/mL, respectively. MH3 and MH7 showed an inhibition zone against *B. subtilis* that displayed MIC values of 2.50 and 5.00 mg/mL, respectively. However, MH3 and MH7 showed no killing effect on *S. aureus.* Furthermore, all extracts showed no effect against *P. aeruginosa*, as shown in Table 5.

Table 5. The MIC (mg/mL) and MBC (mg/mL) of the MH remedy extract.

MH extract	The value of minimum inhibitory concentration and minimum bactericidal concentration					
	S. aureus		*P. aeruginosa*		*B. subtilis*	
	MIC	MBC	MIC	MBC	MIC	MBC
MH1	>5	>5	>5	>5	>5	>5
MH2	>5	>5	>5	>5	>5	>5
MH3	>5	>5	>5	>5	2.50	2.50
MH4	2.50	2.50	>5	>5	1.25	2.50
MH5	>5	>5	>5	>5	>5	>5
MH6	>5	>5	>5	>5	>5	>5
MH7	>5	>5	>5	>5	5	>5
AM	0.195	0.195	2.50	2.50	0.098	0.098

The antibacterial activity of plant ingredient extract of MH remedy is presented in Table 6. All extracts have no inhibitory effect on *P. aeruginosa* and *S. aureus*. However, the TD1 extract showed the best activity to inhibit *B. subtilis,* with an inhibition zone of 19.50 mm, followed by TK2 with the inhibition zone of 17.00 mm. Moreover, TD2 and TK1 have also an inhibitory effect on *B. subtilis*, which has the inhibitory area equal to 10.40 and 8.00 mm, respectively, as shown in Table 6.

Table 6. Antibacterial activities of plant ingredient extract of MH remedy against bacterial test organism.

Plant ingredient extract	Zone of inhibition in mm.		
	S. aureus	*P. aeruginosa*	*B. subtilis*
TD1	NI	NI	19.50±0.30
TD2	NI	NI	10.40±0.29
TK1	NI	NI	8.00±00.23
TK2	NI	NI	17.00±0.29
TK3	NI	NI	NI
DP	NI	NI	NI
AM	28.33±0.17	NI	30.13±0.09

Table 7. The MIC (mg/mL) and MBC (mg/mL) of plant ingredient extract of MH remedy.

plant ingredient extract	The value of minimum inhibitory concentration and minimum bactericidal concentration					
	S. aureus		*P. aeruginosa*		*B. subtilis*	
	MIC	MBC	MIC	MBC	MIC	MBC
TD1	>5	>5	>5	>5	0.5	0.5
TD2	>5	>5	>5	>5	0.5	0.5
TK1	0.5	0.5	>5	>5	0.625	0.625
TK2	0.5	0.5	>5	>5	0.25	0.25
TK3	>5	>5	>5	>5	>5	>5
DP	>5	>5	>5	>5	>5	>5
AM	0.195	0.195	2.5	2.5	0.0976	0.0976

From Table 7, it was found that the extract from TK2 shows the best antibacterial activity against *B. subtilis* with MIC and MBC at 0.25 mg/mL. TD1 and TD2 show antibacterial activity against *B. subtilis* at 0.50 mg/mL, while TK1 and TK2 resisted *S. aureus* bacteria by showing MIC and MBC values at 0.50 mg/mL which is considered acceptable**.** From the tests of all extracts, there is no extract against *P. aeruginosa*.

The Mahajak remedy that is mentioned in the Tamra-Osodphranarai is used to relieve pain and skin infection after injury [8]. Therefore, this research investigated the antibacterial activity of MH remedy extract with various extractions by taking all the components of Mahajak remedy to test. The results show that gram-positive bacteria were more sensitive to the MH extract than gram-negative bacteria. The 95% ethanolic extract of MH remedy (MH4) showed the highest antibacterial activity against *S. aureus* and *B. subtilis* while the hexane extract and the 95% ethanolic extract of hexane residue have little effect against *B. subtilis*.

Moreover, the MH remedy contained *P. retrofractum* as a major ingredient. Thus, the hexane extract of MH remedy may contain non-polar chemical substances from *P. retrofractum* such as piperine and piperanine [14]. Piperine can inhibit *S. aureus* (50 µg/mL) and increase the ability of ciprofloxacin to reduce *S. aureus* [15]. Piperine can be dissolved in the organic solvent [16], and we also found that *F. vulgare* had an antibacterial activity against *S. aureus* and *P. aeruginosa* following the activity against *B. subtilis*. Many plant components of the MH remedy contain an essential oil that can increase the permeability of bacterial cell membranes and induce bacterial cell death [17]. Moreover, plant components of the MH remedy, such as *N. sativa* oil, can inhibit multi-drug resistant *S. aureus* that was isolated from a diabetic's patient wound and can increase the patient's wound healing [18]. The *N. sativa* extract was nearly as effective as the standard drug, mupirocin, and showed no side effect [19]. When compared with the experiments done, it was found that TD1 could inhibit some skin-related bacteria. Previous reports about *P. retrofractum* (DP) showed that the methanolic extract of *P. retrofractum* extract inhibits *S. aureus* and *B. subtililis* with MIC and MBC values of 225 µg/mL [20]. The results of the experiment may be due to DP contains alkaloid compounds such as results of the experiments in this work that found the value of TK2 shows the best antibacterial activity with *B. subtilis* with MIC and MBC at 0.25 mg/mL [21]. There was a research report on *Lepidium sativum* L. (TD2) seed oil that revealed anti-inflammatory and antimicrobial activity against *S. aureus, B. subtilis, P. aeruginosa* [22]. The antimicrobial activity of essential oil from

dill (*Anethum graveolens* L.) from seeds investigated in terms of the disc-diffusion method showed the best antimicrobial activity against *S.aureus* [23]. Moreover, the essential oils from the fruit peel of *Citrus hystrix* DC have antibacterial activity [24]. In this research, the residue of hexane extract is extracted by maceration with 95% ethanol to obtain MH4 extract. It also showed antibacterial activity against *B. subtilis* because it may contain polar substances that are extracted by ethanol. All plants in the MH remedy contain similar components, such as volatile oil, flavonoids, alkaloids, and coumarins. These chemical compounds also show antibacterial and anti-inflammatory activity and reduce muscle pain [25]. However, MH4 showed lower antibacterial activity than its plant ingredients, which may be caused by the proportion of each plant ingredient in MH4 being 14-28%. Thus, MH4 showed low antibacterial activity. Moreover, all of the extracts, including MH and plant ingredients, also showed low antibacterial activity when compared with ampicillin as a positive control. Ampicillin is a pure compound that inhibits pathogens [26]. While MH extract and plant ingredient extracts contained many active compounds that may inhibit bacteria. Active compounds in MH may show good effect on antibacterial activity when it is isolated from plant extracts and MH remedy extracts.

4. Conclusion

In conclusion, our results provided evidence that the MH remedy extract has moderate antibacterial activity. Furthermore, chemical constituents of the Mahajak remedy and biological activities that involve wound healing and pain relief should be performed in the future.

Acknowledgements

We would like to thank the Center of Excellence in Applied Thai Traditional Medicine Research, Faculty of Medicine, Thammasat University, for financial support of this research.

References

[1] Daniel L, Marc A, Richard A. Common Bacterial Skin Infections. Am Fam Physician. 2002;66(1):119-25.

[2] Stulberg DL, Penrod MA, Blatny RA. Common bacterial skin infections. Am Fam Physician 2002;66(1):119-24.

[3] Sukumaran V, Senanayake S. Bacterial skin and soft tissue infections. Australian prescriber 2016;39(5):159-63.

[4] Hogberg L, Heddini A, Cars O. The global need for effective antibiotics: challenges and recent advances. Trends Pharmacol Sci 2010;31(11):509-15.

[5] Sunderkotter C, Becker K. Frequent bacterial skin and soft tissue infections: diagnostic signs and treatment. JDDG. 2015;13(6):501-24.

[6] National Antimicrobial Resistance Surveillance Center Thailand (NARST) . Antimicrobial Resistance Surveillance Center Thailand. Antimicrobial Resistance 2000-2018 [Internet]. [cited 2019 Jul 17]. Available from: http://narst.dmsc.moph.go.th/

[7] Brown DG, Lister T, May-Dracka TL. New natural products as new leads for antibacterial drug discovery. Bioorg Med Chem Lett. 2014;24(2):413-8.

[8] Thai and Alternative Medicine Division. King Narai Hospital. Tamra PhraOsot Phranarai [Brochure]; Lopburi; 2016.

[9] Phichiansunthon C, Chawalit M, Chairawong W. Tamra PhraOsot Phranarai. 1st ed. Bangkok: Amrin and Phoompanya Foundation; 2001.

[10] Ministry of Public Health. Thai herbal pharmacopoeia. Vol. Supplement. Nonthaburi: Department of Medical Sciences, Ministry of Public Health; 2004.

[11] Lorian V. Antibiotics in Laboratory Medicine. 4th ed. Baltimore: Williams&Wilkins; 1996.

[12] Jones RN, Barry AL, Gavan TL, Washington JA. Susceptibility tests: microdilution and microdilution broth procedures. ASM 1985;4:972-7.

[13] Sattaponpan C, Kondo S. Antibacterial activity of crude extracts of Prasaprohyai formula and its components agains pathogenic bacteria. J Med Assoc Thai. 2011;94(Suppl. 7): 153-61.

[14] Wiwattanawanichakun P, Ratwatthanon A, Poonsri W, Yooboon T, Pluempanupat W, Piyasaengthong N, et al. The Possibility of Using Isolated Alkaloid Compoundsnand Crude Extracts of *Piper retrofractum* (Piperaceae)nas Larvicidal Control Agents for Culex quinquefasciatus Diptera: Culicidae) Larvae. J Med Entomol. 2018;55(5):1231-6.

[15] Khan IA, Mirza ZM, Kumar A, Verma V, Qazi GN. Piperine, a phytochemical potentiator of ciprofloxacin against *Staphylococcus aureus*. Antimicrob Agents Chemother. 2006;50(2):810-2.

[16] Namjoyan F, Hejazi H, Ramezani Z. Evaluation of drying process on the composition of black pepper ethanolic extract by high performance liquid chromatography with diode array detector. Jundishapur J Nat Pharm Prod. 2012;7(4):163-7.

[17] O'Bryan CA, Pendleton SJ, Crandall PG, Ricke SC. Potential of Plant Essential Oils and Their Components in Animal Agriculture - in vitro Studies on Antibacterial Mode of Action. Frontiers in Veterinary Science 2015;2:35.

[18] Emeka LB, Emeka PM, Khan TM. Antimicrobial activity of *Nigella sativa* L. seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds. Pak J Pharm Sci. 2015;28(6):1985-90.

[19] Rafati S, Niakan M, Naseri M. Anti-microbial effect of *Nigella sativa* seed extract against *staphylococcal* skin Infection. Med J Islam Repub Iran. 2014;28(42).

[20] Putrifabarni N. Phytochemicals and antibacterial activity of *Piper retrofractum* Vahl. and *Piper arborescens* Roxb.[thesis]. Universiti Teknologi Malaysia 2012.

[21] Agarwal D, Sharma LK, Saxena SN. Anti-microbial properties of fennel (*Foeniculum vulgare* Mill.) seed extract. JPP. 2017;6(4):479-82.

[22] Alqahtania F, Aleanizya F, Mahmoud A, et al. Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of *Lepidium sativum* seed oil. Saudi Journal of Biological Sciences 2018;26(5):1089-92.

[23] Ljiljana P, Mihajlo Z, Dragan J, et al. Dill (*Anethum graveolens* L.) seeds essential oil as a potential natural antioxidant and antimicrobial agent. Biologica Nyssana Journal of Biological Sciences 2016;7(1):31-9.

[24] Srisukha V, Tribuddharatb C, Nukoolkarn V, et al. Antibacterial activity of essential oils from *Citrus hystrix* (makrut lime) against respiratory tract pathogens. J. Sci. Soc. Thailand 2012;38(2):212-7.

[25] Joycharat N. Chemical Constituent in Materia Medica. Prince of Songkla University, Songkla 2018.

[26] Peechakara BV, Basit H, Gupta M. Ampicillin. [Updated 2020 May 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519569/