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Research Article

HERBAL SOAP WITH PIPER BETLE AGAINST *STAPHYLOCOCCUS AUREUS*: EXTRACTION AND FORMULATION

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ABSTRACT

Staphylococcus aureus (S. aureus) is a widespread pathogen responsible for various skin infections globally, regardless of age, climate, or geographic location. As a result, there is growing interest in the development of herbal-based skin cleansing products with antibacterial activity against this bacterium. This study aimed to identify potential medicinal herbs from An Giang Province capable of inhibiting S. aureus. The investigation focused on three herbal extracts—fish mint, bitter melon, and betel leaf—and three essential oils derived from grapefruit peel, tea tree, and betel leaf. The betel leaf extract and essential oil exhibited the strongest antibacterial activity, with minimum inhibitory concentration (MIC) values of 2500 ppm and 6.3 ppm, respectively (compared to 0.2 ppm chloramphenicol as a positive control). The chemical constituents of betel leaf were characterized using LC-MS and GC-MS, identifying 25 compounds in the essential oil and 22 compounds in the extract. Based on these findings, an antibacterial herbal soap formulation containing betel leaf extract and essential oil was successfully developed in accordance with TCVN 2224:1991 standards, highlighting its potential for practical application as a natural antibacterial skin cleanser.

Keywords: antibacterial herbal soap; bitter melon; fish mint; grapefruit; tea tree

1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccus bacterium that commonly colonizes human skin and mucosal surfaces as a human commensal organism. However, under favorable conditions such as the presence of skin abrasions or elevated humidity, it can proliferate and become an opportunistic pathogen, leading to a variety of potentially serious infections (Milani et al., 2023). In the field of dermatology, *S. aureus* is recognized as a primary cause of pyoderma, folliculitis, impetigo, pustules, cellulitis and wound infections (Del Giudice, 2020). In particular, elevated skin humidity creates ideal conditions for the growth of this bacterium in tropical climates because it readily reproduces and penetrates through lesions or wet hair follicles. Its ability to form pus and secrete tissue-damaging toxins further complicates

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the management and treatment of *S. aureus*-associated skin infections (Cheung et al., 2021). Therefore, inhibiting the proliferation of *S. aureus* is essential to prevent serious complications from skin infections (Chambers & DeLeo, 2009). Consequently, identifying bioactive antibacterial compounds from medicinal herbs is a promising strategy for developing safer and more effective skin care products.

In an effort to screen medicinal herbs for antibacterial activity against *S. aureus,* five medicinal herbs were selected for investigation, including grapefruit peel, tea tree, fish mint, bitter melon, and betel leaf. In traditional medicine, these herbs are also widely used to treat skin diseases, soothe lesions, and reduce inflammation and swelling (Do, 2001). Previous studies have also demonstrated their strong antibacterial activity and potential to support the treatment of skin conditions, with low MIC values against many types of bacteria that cause skin infections (Chomnawang et al., 2005; Hoang et al., 2022; Saeed & Tariq, 2005; Tao & Liu, 2012). However, their activity against *S. aureus* has not been explored.

In this study, plant samples were extracted and examined for their antibacterial activity against *S. aureus:* three extracts of fish mint, bitter melon, and betel leaf and three essential oils from grapefruit peel, tea tree, and betel leaf. The results showed that the betel leaf ethanolic extract and essential oil exhibited the strongest antibacterial activity against *S. aureus*, providing a basis for formulating an antibacterial soap containing both. In addition, the chemical composition of these extracts and essential oils was determined by GC-MS and LC-MS, providing a basis for product development.

2. Materials and methods

2.1. Materials

Fresh samples of **grapefruit (***Citrus maxima***) peel, fish mint (***Houttuynia cordata***)**, **bitter melon (***Momordica charantia***)**, **tea tree (***Melaleuca cajuputi***)** and **betel leaf (***Piper betle***)** were collected from An Giang Province, Vietnam. **Coconut oil** and **soybean oil** were obtained from Vietcoco and Tuong An Company, respectively.

All analytical-grade solvents and reagents including **diethyl ether, ethanol, glycerin, citric acid, sodium sulfate (Na_2SO_4), phenolphthalein, hydrochloric acid,** and **dimethyl sulfoxide (DMSO)** were purchased from Xilong (China) and Merck (Germany). **Chloramphenicol** was used as a positive control antibiotic.

Microbiological testing used *Staphylococcus aureus* strain ATCC 6538 and **Mueller-Hinton (MH) agar.** Instruments included a **steam distillation unit, Soxhlet extractor, rotary vacuum evaporator, UV-Vis spectrophotometer, 96-well microplate reader, gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS),** and standard labware.

2.2. Plant extracts and essential oils

2.2.1. Essential oil extraction

Dried plant materials (500 g each) were chopped, ground, and mixed with distilled water at a 1:5 (w/v) ratio in a 2,000 mL round-bottom flask. Steam distillation was carried

out for 4 hours. The essential oils were collected using a separatory funnel, extracted with diethyl ether, dried over anhydrous Na_2SO_4, and concentrated using a rotary evaporator under reduced pressure. The essential oil yield was calculated using Eq. (1):

Extraction yield (%) = (Weight of essential oil / Weight of raw material) × 100 (1)

2.2.2. Ethanolic extract preparation

For each sample (fish mint, bitter melon and betel leaf), 50 g of dried material was extracted with 200 mL of 96% ethanol using ultrasonic-assisted extraction for 2 hours. This procedure was repeated four times. The combined extracts were filtered, concentrated under reduced pressure and freeze-dried to obtain powdered extracts. The yield of ethanolic extract was determined by Eq. (2):

Extraction yield (%) = (Mass of dried extract / Mass of raw material) × 100 (2)

2.2.3. Evaluation of antibacterial activity

Agar Well Diffusion Assay

S. aureus was cultured in MH broth at 37°C for 24 hours and standardized to 10^6–10^7 CFU/mL (measured at OD_{660} nm). MH agar plates were inoculated with the bacterial suspension. Wells of 8 mm diameter were punched and filled with 40 µL of test solution at various concentrations in DMSO. Chloramphenicol (10 mg/mL) was used as the positive control and DMSO as the negative control. After 24 h of incubation, the inhibition zone diameter (DIZ) was measured in millimeters. Each test was performed in triplicate.

Minimum Inhibitory Concentration (MIC)

MIC values were determined using a broth microdilution method in 96-well plates. Each well contained 100 µL of MH broth and 100 µL of diluted test solution in DMSO. Plates were incubated at 37°C for 16–24 hours. Next, 20 µL of 0.01% resazurin solution was added to each well. A color change from blue to pink indicated bacterial growth. The MIC was defined as the lowest concentration at which the solution remained blue. All tests were conducted in triplicate.

2.2.4. Herbal soap formulation

Soap base preparation:

A hot saponification method was used. A mixture of **30 g of coconut oil** and **20 g of soybean oil** was heated and stirred. A **30% NaOH solution (8.20 g)** was slowly added under continuous stirring. The mixture was maintained at 70–80°C in a water bath until a thick, homogeneous soap base formed. pH was adjusted using citric acid if needed. The soap was poured into molds and allowed to solidify at room temperature.

Incorporation of herbal ingredients: The prepared soap base was melted using a double boiler. **A total of 0.08 g of dried betel leaf extract** and **0.75 g of betel leaf essential oil** were added and thoroughly mixed. **A total of 10 mL of distilled water** was added to improve homogeneity. The final mixture was poured into molds and left to solidify at room temperature for 72 hours.

2.2.5. Evaluation of the physicochemical properties of soap bars according to **TCVN 2224:1991**

The physicochemical properties of the soap bars were evaluated according to Vietnamese Standard, TCVN 2224:1991, Bar soap - Technical requirements.

Table 1. *Physicochemical properties of soap bars*

Standard Name	Standard
Content of NaOH, in % of nominal mass, not more than	0.05
Sodium chloride content, in % of nominal mass, not more than	0.82
Content of unsaponifiable fats, in % by mass of fatty acids, not more than	1.00
Content of unsaponifiable organic matter, in % by weight of fatty acids, not more than	1.50
Freezing point of fatty acids separated from soap, in % by mass of fatty acids, not less than	35
Iodine index, expressed in grams of iodine in 100 grams of fatty acids, not more than	45
Initial foam volume of 10.5 % soap solution, in mL, not less than	470

3. Results and discussion

3.1. Extraction yield

Essential oils. All essential oils obtained through steam distillation had a bright appearance, were free of impurities, and had characteristic aromas corresponding to the respective medicinal herbs. The yields of grapefruit, tea tree, and betel leaf essential oils are shown in Table 2. Differences in extraction yield were mainly attributable to the amount of essential oil in each plant species and the parts used in the distillation.

Table 2. *Essential oil extraction yield by steam distillation method*

Essential Oils	Parts used	Weight of ingredients (g)	Essential oil mass (g)	Efficiency (%)
Grapefruit	Peels	1621.82	33.81	2.08
Tea tree	Leaves and branches	3963.08	62.29	1.57
Betel	Leaves	2342.51	11.70	0.50

Extracts. After the extraction process, solvent evaporation and drying, all extracts obtained were homogeneous and had pleasant aromas. The ethanolic extraction yields of the samples are shown in Table 3. The extraction yields of dried samples were higher than those of fresh samples. This difference was primarily attributable to the high water content of fresh plant materials, which diluted the concentration of extractable compounds.

Table 3. *Extraction yield of ethanolic extracts*

Ethanolic extracts	Sample mass (g)	Mass ethanol (g)	Effect rate (%)
Fish mint	102.1	3.4	3.4
Bitter melon	169.2	5.2	3.1
Betel leaf	118.7	4.6	3.9

3.2. Antibacterial activity of extracts and essential oils against S. aureus

The results of the antibacterial activity assays (Table 4) showed that all extracts and essential oils effectively inhibited the growth of *S. aureus.*

Table 4. *Antibacterial activity of the ethanolic extracts (EEs) against S. aureus at a concentration of 5 mg/well for extract samples and 20 µL/well for essential oil (EOs)*

Sample (5 mg /well)	**Diameter of inhibition zone (mm)** ***S. aureus* ATCC 6538**
Fish mint EE	10.0 ± 0.2
Bitter melon EE	9.0 ± 0.2
Betel leaf EE	13.5 ± 0.1
Grapefruit EO	12.0 ± 0.2
Tea tree EO	15.0 ± 0.2
Betel EO	20.0 ± 0.1
Chloramphenicol[a] (20 μg)	27.0 ± 0.1

[a] *Positive control*

The MIC results for the antibacterial activity of the extracts against *S. aureus* (Table 5) were consistent with the results from the agar well diffusion assay. Compared with the positive control chloramphenicol, the extracts exhibited weaker antibacterial activity against *S. aureus*. These essential oils showed moderate antibacterial activity compared to the positive control chloramphenicol (0.2 ppm).

Table 5. *Minimum inhibitory concentration (MIC) of the extracts and essential oils against S. aureus*

Sample	**MIC (ppm)** ***S. aureus* ATCC 6538**
Fish mint EE	7500.0
Bitter melon EE	10000.0
Betel leaf EE	2500.0
Grapefruit EO	17.5
Tea tree EO	15.0
Betel EO	6.3
Chloramphenicol[a]	0.2

[a] *Positive control*

The antibacterial activity assay demonstrated that fish mint, bitter melon, dried betel leaf, tea tree oil, grapefruit oil, and betel leaf essential oil all could inhibit the growth of *S. aureus.* Betel leaf extract and betel leaf essential oil were selected as antibacterial ingredients for the soap products.

3.3. Chemical composition of essential oils and extracts of Betel leaf

Chemical composition of Betel essential oil: The GC-MS results (Table 6) showed that approximately 25 compounds were identified in the betel leaf essential oil obtained by steam distillation. The structures of the compounds are shown in Figure 1.

Table 6. *Chemical composition of Betel leaf essential oil obtained by steam distillation*

No.	Compound	Formula	R_t	*m/z*
1	*p*-Cresidine	$C_8H_{11}NO$	9.182	122
2	Eucalyptol	$C_{10}H_{18}O$	9.813	43
3	β-Thujene		7,684; 9.733	93
4	γ-Terpinene	$C_{10}H_{16}$	10.768	93
5	α-Terpinene		9,195; 11.877; 23.058	121
6	α-Pyronene		22.906	121
7	Linalool	$C_{10}H_{18}O$	12.463	71
8	(-)-Terpinen-4-ol		16.103	71
9	α-Terpinyl formate	$C_{11}H_{18}O_2$	16.765	59
10	Eugenol	$C_{10}H_{12}O_2$	23.833	164
11	α-Cubebene		24.901	161
12	Germacrene B		25.187	93
13	β - Bourbonene		25.261	81
14	β-Elemene		25.511	93
15	β-*cis*-Caryophyllene		26.847	93
16	γ-Cadinene	$C_{15}H_{24}$	29.254;30.889	161
17	δ-Cadinene		31.088	161
18	Cadina-3,5-diene		30.445	161
19	Epizonarene		31.292	161
20	α-Muurolene		30.263	105
21	Cubenene		31.693	119
22	α-Cadinol		36.744	95
23	Nerolidol 2	$C_{15}H_{26}O$	32.883	69
24	*tau*.-Muurolol		36.267	95
25	Ethanone	$C_9H_8O_3$	34.802	149

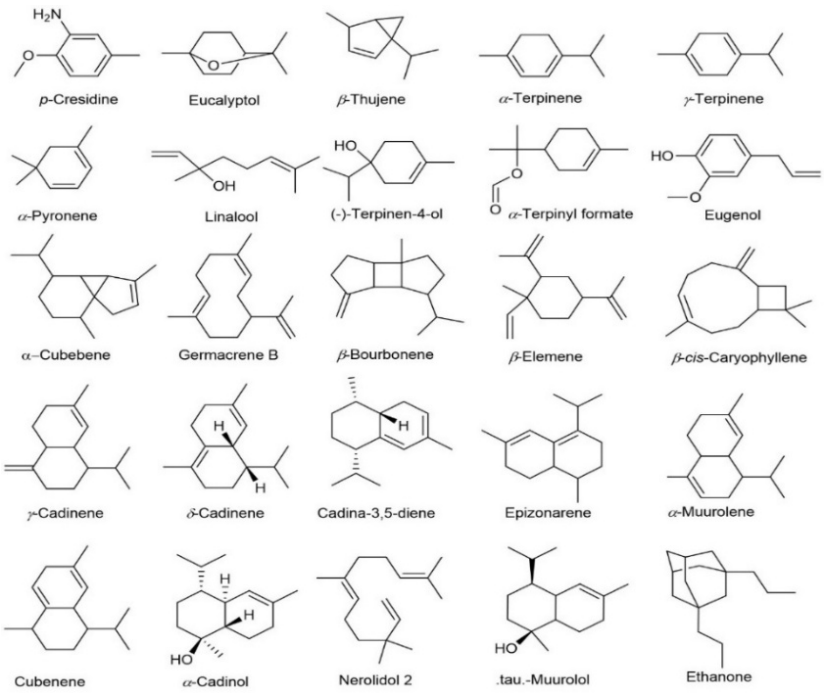

Figure 1. *Structure of identified compounds in Betel essential oil*

Chemical composition of Betel leaf extract: The ethanolic extract obtained by ultrasound-assisted extraction was analyzed by liquid chromatography-mass spectrometry (LC-MS) (Table 7), and 22 compounds were identified (Figure 2). The structures of the compounds are shown in Figures 3 and 4.

Table 7. *Chemical composition of Betel leaf extract obtained by ultrasonic methods*

No.	Compound	Formula	R_t	*m/z*
1	Vitamin B5	$C_9H_{17}NO_5$	1.25	278.1252
2	5-Oxoproline	$C_5H_7NO_3$	1.50	128.0359
3	Succinic acid	$C_4H_6O_4$	1.70	117.0197
4	DL-Tyrosine	$C_9H_{11}NO_3$	1.80	180.0671
5	Uridine	$C_9H_{12}N_2O_6$	1.94	289.0685
6	Pantothenic acid	$C_{11}H_{21}NO_5$	2.75	292.1407
7	Adenosine	$C_{10}H_{13}N_5O_4$	3.26	312.0957
8	L-Phenylalanine	$C_9H_{11}NO_2$	4.27	164.0723
9	Sesamol	$C_7H_6O_3$	7.26	137.0248
10	Caffeic acid 3-glucoside	$C_{15}H_{18}O_9$	8.48	341.0887
11	Isorhamnetin-3-O-β-gentiobioside	$C_{28}H_{32}O_{17}$	9.11	639.1586
12	Complanatuside	$C_{28}H_{32}O_{16}$	9.31	623.1639
13	Sophorabioside	$C_{27}H_{30}O_{14}$	9.32	577.1587
14	2,5,7-Trihydroxy-6,8-dimethyl-3-(4'-methoxybenzyl) chroman-4-one	$C_{19}H_{20}O_6$	10.90	343.1202
15	Phenethyl ferulate	$C_{18}H_{18}O_4$	11.43	297.1148
16	Denudatin A	$C_{20}H_{20}O_5$	11.67	339.1259
17	γ-Linoleic acid	$C_{18}H_{30}O_2$	12.87	277.2184
18	Ethyl palmitoleate	$C_{18}H_{34}O_2$	13.18	281.2500
19	Behenic acid	$C_{22}H_{44}O_2$	14.23	339.3278
20	Tetracosanoic acid	$C_{24}H_{48}O_2$	14.83	367.3594
21	Pentacosanoic acid	$C_{25}H_{50}O_2$	15.73	441.3951
22	Cerotic acid	$C_{26}H_{52}O_2$	15.74	395.3905

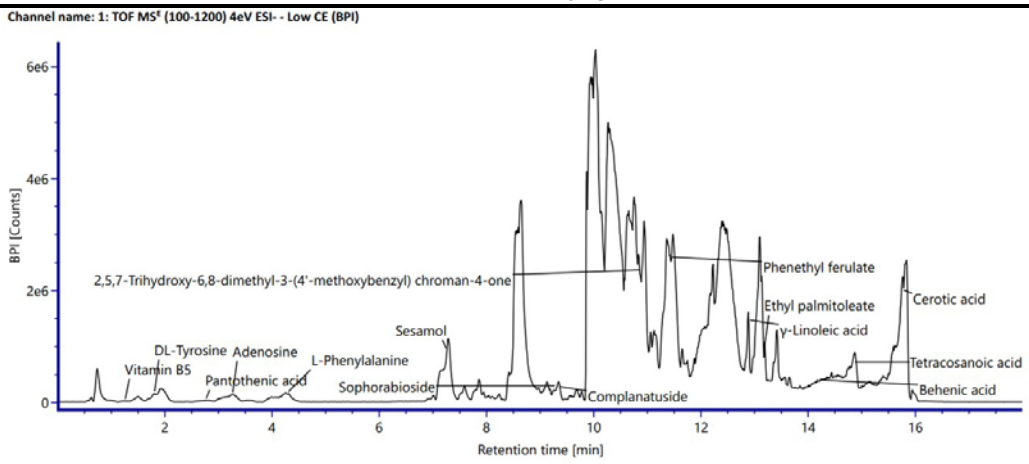

Figure 2. *Chromatogram of Betel extract*

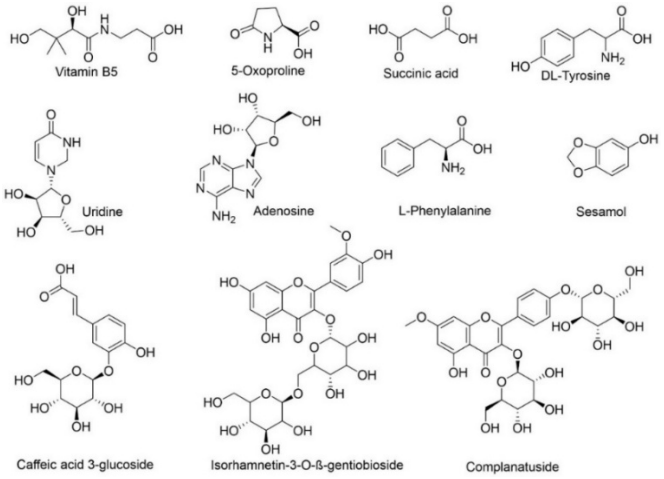

Figure 3. *Structure of identified compounds in Betel ethanolic extract*

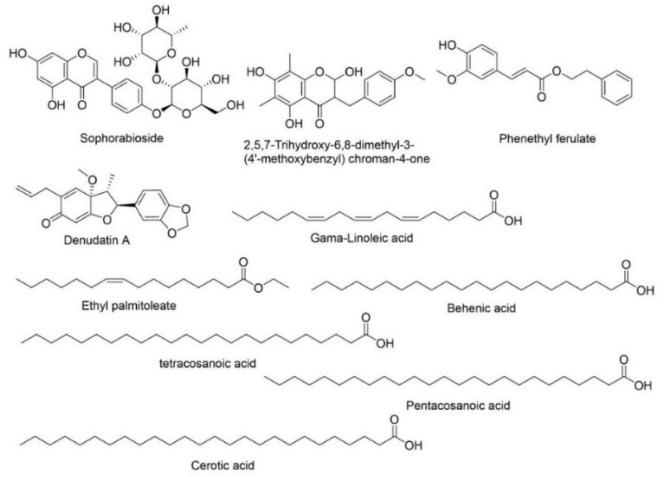

Figure 4. *Structure of identified compounds in Betel ethanolic extract (next)*

3.4. Betel leaf soap

The betel leaf soap possessed a hard, firm, and smooth texture, without cracks (Figure 5). The color of the antibacterial herbal soap sample was bright and uniform and matched the color of the betel leaf extract. The soap had a pleasant aroma, characteristic of betel leaf essential oil, with no odor of rancid oil. During use tests, the soap effectively cleansed the skin, leaving the treated areas feeling soft and refreshed, accompanied by a pleasant fragrance.

Figure 5. *Antibacterial herbal soap*

3.5. Physical and chemical properties of soap bars according to TCVN 2224:1991

The results of the physicochemical evaluation of the soap bars according to TCVN 2224:1991, Bar soap - Technical requirements, are shown in Table 8.

Table 8. *Physicochemical properties of the soap bar sample*

Standard Name	Standard	Sample
Content of NaOH, in % of nominal mass, not more than	0.05	0
Sodium chloride content, in % of nominal mass, not more than	0.82	0
Content of unsaponifiable fats, in % by mass of fatty acids, not more than	1.00	0.51
Content of unsaponifiable organic matter, in % by weight of fatty acids, not more than	1.50	1.10
Freezing point of fatty acids separated from soap, in % by mass of fatty acids, not less than	35	38
Iodine index, expressed in grams of iodine in 100 grams of fatty acids, not more than	45	43
Initial foam volume of 10.5 % soap solution, in mL, not less than	470	913

4. Conclusion

Taken together, the results showed that both the herbal extracts and essential oils exhibited antibacterial activity against *S. aureus*. In particular, Betel leaf demonstrated the strongest antibacterial activity, with MIC values of 2500 ppm for the extract and 6.5 ppm for the essential oil, respectively. Chemical analysis by LC-MS and GC-MS identified the principal compounds: monoterpenes and sesquiterpenes in the essential oil and flavonoids in the betel leaf extract. These compounds may help explain the bioactivity of this herb. In addition, an antibacterial herbal soap was successfully produced using betel leaf extract and essential oil in accordance with TCVN 2224:1991; it had a pleasant feel and natural fragrance, supporting its use as a natural and safe skin cleanser. These results not only confirm the potential for practical application of local and traditional herbs in antibacterial products but also provide a basis for further studies on the antibacterial activity of bioactive compounds in betel and other herbs.

❖ ***Conflict of Interest:*** *Author have no conflict of interest to declare.*

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CHIẾT XUẤT VÀ BÀO CHẾ XÀ PHÒNG THẢO DƯỢC TỪ LÁ TRẦU KHÔNG VỚI HOẠT TÍNH KHÁNG *STAPHYLOCOCCUS AUREUS*

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TÓM TẮT

Staphylococcus aureus (S. aureus) là tác nhân gây bệnh phổ biến, gây ra nhiều loại nhiễm trùng da trên toàn thế giới, không phân biệt độ tuổi, khí hậu hay khu vực địa lí. Do đó, việc nghiên cứu phát triển các sản phẩm làm sạch da có nguồn gốc thảo dược với hoạt tính kháng khuẩn đối với vi khuẩn này ngày càng được quan tâm. Nghiên cứu này nhằm xác định các loài dược liệu tiềm năng tại tỉnh An Giang có khả năng ức chế S. aureus. Thí nghiệm khảo sát ba loại dịch chiết thảo dược gồm diếp cá, mướp đắng và lá trầu không, cùng ba loại tinh dầu gồm vỏ bưởi, tràm trà và lá trầu không. Cả dịch chiết và tinh dầu lá trầu không đều thể hiện hoạt tính kháng khuẩn mạnh nhất với nồng độ ức chế tối thiểu (MIC) lần lượt là 2500 ppm và 6,3 ppm (so với 0,2 ppm chloramphenicol làm đối chứng dương). Thành phần hóa học của lá trầu không được phân tích bằng LC-MS và GC-MS, xác định 25 hợp chất trong tinh dầu và 22 hợp chất trong dịch chiết. Trên cơ sở đó, công thức xà phòng thảo dược chứa dịch chiết và tinh dầu lá trầu không đã được xây dựng thành công theo tiêu chuẩn TCVN 2224:1991, cho thấy tiềm năng ứng dụng thực tiễn như một sản phẩm làm sạch da có nguồn gốc tự nhiên và khả năng kháng khuẩn.

Từ khóa: xà phòng thảo dược kháng khuẩn; bưởi; diếp cá; mướp đắng; lá trầu không