International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 20s, 2025
https://theaspd.com/index.php

Antibacterial Activity Against Skin Pathogenic Bacteria Using Thailand Medicinal Plants

Sumonthip Kongtunjanphuk[1]
[1]Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand
[1]sumonthip.k@sci.kmutnb.ac.th
*Corresponding Author: sumonthip.k@sci.kmutnb.ac.th

***Abstract*—** *This study investigated the antibacterial activity of crude extracts from five medicinal plants, including Cassia alata (L.). Roxb. (Ringworm Bush), Curcuma longa L. (Turmeric), Lawsonia inermis L. (Henna), Rhinacanthus nasutus (L.) Kurz. (White Crane Flower), and Sapindus emarginatus Wall. (Soap Nut Tree)–against three skin pathogenic bacteria: Pseudomonas aeruginosa TISTR 1467, Staphylococcus aureus TISTR 118, and Staphylococcus epidermidis TISTR 1845. Two types of crude extracts, namely aqueous and ethanol extracts, were tested. The results revealed that only the aqueous extract of Sapindus emarginatus showed all three bacterial antibacterial activity. However, the other aqueous extracts of the different plants showed no antibacterial activity. In contrast, ethanol extracts exhibited antibacterial effects across all medicinal plants, with the most effective activity observed in the ethanol extracts of Rhinacanthus nasutus and Sapindus emarginatus. These crude extracts can be further developed into phytopharmaceutical products, including shampoos, soaps, or body cleansing agents with antibacterial activity to inhibit bacteria-causing skin infections.*

***Keywords*— Antibacterial, Extraction, Medicinal plants, Skin pathogenic bacteria.**

I. INTRODUCTION

Consumers' interest in phytopharmaceutical products has surged in recent years [1], driven by a growing awareness of health and safety. The preference for natural products, known to have fewer side effects than synthetic chemicals, has further fueled this trend. With their therapeutic benefits and safety profile, phytopharmaceuticals have emerged as a reassuring option for individuals seeking effective yet safe products. Producing high-quality phytopharmaceuticals, supported by advancements in science and technology, has only enhanced consumer trust in their safety and effectiveness. As a result, the global phytopharmaceutical market has expanded rapidly [2]-[3].

With abundant natural resources, Thailand is a source of medicinal plants with immense potential for phytopharmaceutical development [4]-[5]. These include Cassia alata (L.) Roxb., known as Ringworm bush, is a potent treatment for skin conditions [6]-[8]; Lawsonia inermis L., or Henna, a popular ingredient in hair care products [9]; Rhinacanthus nasutus (L.) Kurz., or White Crane Flower, with its antifungal and antibacterial properties [10]; Sapindus emarginatus Wall., or Soap Nut Tree, a key component in natural cleaning products [11]; Curcuma longa L., or Turmeric, is celebrated for its antioxidant and anti-inflammatory properties [12]. These plants, deeply rooted in traditional Thai medicine, are now the focus of modern research to develop extracts and new products to meet the global demand for phytopharmaceuticals.

A literature review highlights the active compounds extracted from these plants, which exhibit antimicrobial properties against bacteria associated with skin diseases. The extraction process involves an aqueous and ethanol extract, isolating a bioactive compound. For instance, Cassia alata is reported to contain anthraquinones with potent antibacterial activity [13]-[14]. Curcuma longa contains curcumin, a compound with anti-inflammatory and antibacterial properties [15]. Lawsonia inermis is rich in lawsone, which has antimicrobial properties [16], whereas Rhinacanthus nasutus contains rhinacanthin, which is effective against bacteria and fungi [17]. Sapindus emarginatus is abundant in saponins, which help reduce bacterial adhesion to the skin [18]. These findings highlight the potential of Thai medicinal plants for developing antimicrobial agents to treat skin infections.

This study focuses on evaluating the antibacterial efficacy of crude extracts from five medicinal plants: Cassia alata (Ringworm Bush), Curcuma longa (Turmeric), Lawsonia inermis (Henna), Rhinacanthus nasutus (White Crane Flower), and Sapindus emarginatus (Soap Nut Tree). The targeted skin pathogenic

bacteria include Pseudomonas aeruginosa TISTR 1467, Staphylococcus aureus TISTR 118, and Staphylococcus epidermidis TISTR 1845. The aim is to explore their potential for further development into various phytopharmaceutical products, such as shampoos with enhanced antibacterial properties, soaps that can effectively inhibit bacterial growth, or other formulations capable of treating skin-related infections.

II. MATERIALS AND METHODS

A. Plants

This study utilized fresh Thai medicinal plants from various parts of five plant species collected from Lopburi Province, Thailand. Each species was used in fresh form for aqueous extraction and dried for ethanol extraction. The plant species used in this study include Cassia alata (L.) Roxb. (Ringworm Bush), using the leaves; Lawsonia inermis L. (Henna), using the leaves, flowers, fruits, bark, and roots; Rhinacanthus nasutus (L.) Kurz. (White Crane Flower), using the leaves; Sapindus emarginatus Wall. (Soap Nut Tree), using the fruits and Curcuma longa L. (Turmeric), using the rhizomes.

B. Plants Preparation

The herbal preparation was separated into cleaning and medicinal plant preparation for extraction. Part 1, the cleaning procedure of medicinal plants for the subsequent experiments, involves thoroughly cleaning each plant to remove dust, soil, weevils, and insects. After washing, the plants are cut or reduced in size and then air-dried.

Part 2, the fresh plants from Part 1, can be used directly to prepare an aqueous extraction. However, for the preparation of plants for ethanol extraction, follow the steps below:

After washing the plants described in Part 1, the finely cut plants are dried in a hot air oven (Memmert, Universal oven UN30m, Germany) at 60°C for 8 hours. The dried herbs are then ground into a fine powder that can pass through a 30-mesh sieve (RETSCH® AS200 BASIC, Germany) or smaller than 600 µm, as shown in Fig. 1. The powdered plants are tested for moisture content.

In this experiment, the powdered plants' moisture content must not exceed 10% before being used for the extraction process in the following procedure. The moisture content is analyzed by weighing 2 g of each ground plant and testing it with a moisture analyzer (AND MX-50, Japan) at 105°C. If the moisture content exceeds 10%, the plants are re-dried at 60°C for another 8 hours and retested until the moisture content falls below 10%.

Cassia alata | Lawsonia inermis | Rhinacanthus nasutus | Sapindus emarginatus | Curcuma longa

Fig. 1 Characteristics of each grounded medicinal plant

C. Extraction Procedure

1) Aqueous extraction

The fresh plants weighed 500 g and were placed into a glass container. The 2,500 ml of distilled water (at a 1:5 herb-to-water ratio) was added until the herbs were fully submerged. The mixture was boiled at 100°C for 30 minutes to extract the active compounds from each plant. The extract was left to cool down and then coarsely filtered using a muslin cloth once to remove plant residues. Then, a crude extract was filtered twice by cotton.

Next, the crude extract was poured into an evaporating basin (Haldenwanger, Germany) and placed in a water bath (MEMMERT WNB22, Germany). The crude extract was then evaporated at 95°C to achieve the solvent completely evaporating. As a result, a concentrated crude extract was stored in a glass container with a tight lid.

2) Ethanol extraction

The powdered plants weighed 500 g (according to Section B Part 2). Ensured the moisture content did not exceed 10%, the herbs were placed into a tightly sealed glass container. Add 2,500 ml of 95% ethanol

(at a 1:5 herb-to-solvent ratio) to reach fully submerged of the powdered plant. The mixture was then macerated for 7 days, stirring the herbs in the solvent daily.

Afterward, remove the solvent from the crude extract using a rotary evaporator (BUCHI, Japan) at 60°C and 74.51 torr pressure. This step evaporates most of the solvents but not entirely. Transfer the partially concentrated extract into an evaporating basin and further evaporate the remaining solvent in a water bath at 95°C until fully evaporated. The resulting extract will be thick and viscous. Store the extract in a tightly sealed glass bottle for the following experiment phase.

D. Qualitative Analysis of the Extraction

1) Stationary phase preparation

This study used silica gel 60 GF_{254} plates (Merck, Germany) as the stationary phase for analyzing the identity of various crude extracts through Thin-Layer Chromatography (TLC). These aluminum plates measured 20 x 20 cm. Each crude extract (10 mg) was dissolved in 1 ml of methanol and placed into a microcentrifuge tube (Eppendorf, Germany). The mixture was sonicated using a sonicator bath (BRANSON 2510, United States of America) at 40 kHz for 15 minutes. The resulting solution was centrifuged using a centrifuge (Eppendorf, Germany) at 10,000 rpm for 5 minutes. The supernatant was spotted in the stationary phase using a capillary tube for TLC analysis.

For High-Performance Thin-Layer Chromatography (HPTLC), the crude extract samples were applied onto the stationary phase using a TLC semi-automatic sampler (CAMAG® Linomat 5, Switzerland).

In addition to crude extract, standard markers such as Rutin and Charantin were also applied. These standard compounds evaluated the extracts' antibacterial effect against skin pathogenic bacteria and served as standard markers.

2) Stationary phase development by iodine vapor

Iodine crystals were placed 1-5 g into a TLC tank (CAMAG® Flat Bottom Chamber, Switzerland) and sealed the lid tightly. The iodine sublimated in the chamber and became saturated with pink iodine vapor. Once it reached saturation, the stationary phase was inserted into the tank, and the lid was resealed tightly. The stationary phase reacts in the tank, and its surface turns brown. After this reaction is complete, proceed with further analysis.

3) Stationary phase development by spraying method

For the analysis, spray 10% v/v sulfuric acid solution (Merck, Germany) onto the stationary phase. This solution is prepared by diluting 5 ml of concentrated sulfuric acid in 45 ml of 95% ethanol using a 50 ml volumetric flask. After spraying, place the stationary phase in an oven at 105°C for 5 minutes to accelerate the reaction of the crude extracts. Once the response is complete, proceed with further analysis.

4) Mobile phase preparation

This study applied two mobile phase systems to separate compounds in crude extracts. System 1 consisted of dichloromethane: methanol (Merck, Germany) in a ratio of 9:1, whereas System 2 consisted of Dichloromethane: methanol: water: acetic acid in a ratio of 15: 7: 1: 0.1. These systems were selected to effectively separate the compounds in each crude extract, as different compounds may exhibit varying affinities for specific solvent systems.

5) TLC analysis

The stationary and developed stationary phases were immersed in each mobile phase system to separate the compounds and identify the crude extracts. The separation results were visually observed under UV light at 254 nm and 366 nm using the visualizer TLC (CAMAG® Visualizer 3, Switzerland) and the rate of flow (R_f) values were calculated using (1).

$$R_f = \frac{\text{distance of the sample}}{\text{distance of the solvent}} \quad (1)$$

E. Antibacterial Activity

1) Nutrient preparation

Nutrient Broth (NB) is a liquid medium used to cultivate bacteria for various experiments and to determine the maximum bacterial growth. To prepare, 13 g of NB powder was weighed and dissolved in 1,000 ml of distilled water. The liquid was then stirred to achieve a homogeneous solution. Then, 100 ml of the solution was separated into a 250 ml Erlenmeyer flask, covered with cotton, and sterilized in an

autoclave (HIRAYAMA, HICLAVE HVA-110, Japan) at 121°C and 15 psi for 15 minutes. It was allowed to cool before being stored for the following procedures.

Plate Count Agar (PCA) is a solid medium used to grow bacteria and determine bacterial cell count through the Agar Diffusion Method. To prepare, weigh 23.5 g of PCA powder and dissolve it by heating it in 1,000 ml of distilled water. The solution was stirred to achieve homogeneity. Then, transfer the solution into a 2,000 ml reagent bottle and sterilize it in the autoclave at 121°C and 15 psi for 15 minutes. After cooling to 45-50°C, pour 20-25 ml of PCA into sterile 15x100 mm Petri dishes (Anumbra, Czech). Allow the PCA to solidify under UV light in a laminar airflow cabinet (FASTER BHA48, Italy) and store it for the following procedures.

Mueller Hinton Agar (MHA) is a solid medium that tests various extracts' minimum inhibitory concentration (MIC). The MHA weighed 38 g and was dissolved by heating it in 1,000 ml of distilled water. The liquid medium was stirred to achieve homogeneity. Then, the solution was transferred into the 2,000-ml reagent bottle and sterilized in the autoclave at 121°C and 15 psi for 15 minutes. After cooling to 45-50°C, pour 20-25 ml of MHA into the sterile Petri dishes. Allow the MHA to solidify under UV light in the laminar airflow cabinet and store it for further use.

2) Bacteria preparation

Three skin pathogenic bacteria are Pseudomonas aeruginosa TISTR 1467, Staphylococcus aureus TISTR 118, and Staphylococcus epidermidis TISTR 1845. Each bacteria strain was cultured separately in the prepared NB medium described above. The process started by pipetting 1 ml of the bacterial liquid stock culture into an Erlenmeyer flask containing 100 ml of NB. The flask was then incubated with shaking at 250 rpm at 37°C for 24 hours. The bacterial culture was stored for the subsequent experimental procedures.

3) Growth rate determination

This experiment aims to determine the optimal growth rate of each bacterial strain. A 1-ml sample of each bacteria strain from the above section was transferred into a new Erlenmeyer flask containing 100 ml of NB. The flask was incubated with shaking at 250 rpm at 37°C. Samples were taken hourly, with 0.75 ml of the cultural bacteria transferred into a quartz cuvette for absorbance measurement at 600 nm using a spectrophotometer (JENWAY 7200, United Kingdom). The highest absorbance is the maximum growth that occurred for each bacteria strain, which can distribute the period for cultivation on the subsequent procedure.

4) Agar diffusion method

This experiment aims to determine the antibacterial activity of crude extracts by observing the inhibition zone. Initially, bacteria cell suspensions are diluted using the Broth Dilution Method to obtain a bacterial concentration within the 30 to 300 CFU/ml range. This is achieved by diluting 1 ml of bacterial culture into 9 ml of sterile distilled water (10-fold dilution) and repeating to reach a final dilution of 10^{-10}. Then, 100 µl from each dilution (10^{-1} to 10^{-10}) is spread onto PCA plates using a sterile glass spreader. The plates are incubated at 37°C for 18 hours, and the colonies are counted using a hemocytometer (Menzel-Gläser Cubreobjetos, Germany).

5) Quantitative analysis

Control Group (McFarland Standard No. 0.5): This is used as a benchmark to compare the antibacterial activity of the crude extracts. The various quantities of McFarland Standard No. 0.5 (10, 20, and 30 µl) are applied to sterile 6 mm membrane filter discs on MHA plates inoculated with the bacteria.

Aqueous extract: The crude aqueous extracts are diluted to a concentration of 10 mg/ml. 10, 20, and 30 µl Volumes are applied to discs placed on MHA plates inoculated with bacteria to test for inhibitory effects.

Ethanol extract: The crude ethanol extracts are dissolved in Dimethyl sulfoxide (DMSO) to a final concentration of 1 mg/ml. Similarly, the crude aqueous extracts, different volumes (10, 20, and 30 µl) of the crude ethanol extract are applied to discs on MHA plates for antibacterial testing.

The inhibitory zone around each disc is measured to determine the most effective concentration of each crude extract, which will then be used in the Minimum Inhibitory Concentration (MIC) testing.

6) Maximum inhibitory concentration (MIC)

The MIC test aims to identify the lowest concentration of each crude extract for its antibacterial activity or the efficiency of the bacterial growth inhibitory effect. The process begins with cultivating bacteria to their maximum growth rate. Subsequently, the dilution factors are adjusted using the Agar Diffusion

METHOD.
Once the appropriate bacterial concentration (30-300 CFU/ml) is reached, the bacterial suspension is combined with each crude extract at a 10 mg/ml concentration. This mixture is then serially diluted to a concentration of 10^{-10} and spread onto Plate Count Agar. Finally, the number of viable cells is counted to determine the MIC, representing the minimal concentration of the crude extract that successfully shows an antibacterial property.

III. RESULTS

A. Quantitative Analysis of Extraction

The extracts from each herb exhibited colors characteristic of their respective sources. Notably, the Sapindus emarginatus Wall. Yielded the highest extraction quantity in both methods, as shown in Table I and Fig. 2, with yields ranging between 43% and 45%.

TABLE I
YIELD OF THE EXTRACTION

Medicinal plants	%Yield of the extraction	
	Aqueous extracts	Ethanol extracts
Cassia alata	12.84	9.10
Lawsonia inermis	12.10	2.80
Rhinacanthus nasutus	6.60	3.25
Sapindus emarginatus	43.14	44.52
Curcuma longa	3.94	29.85

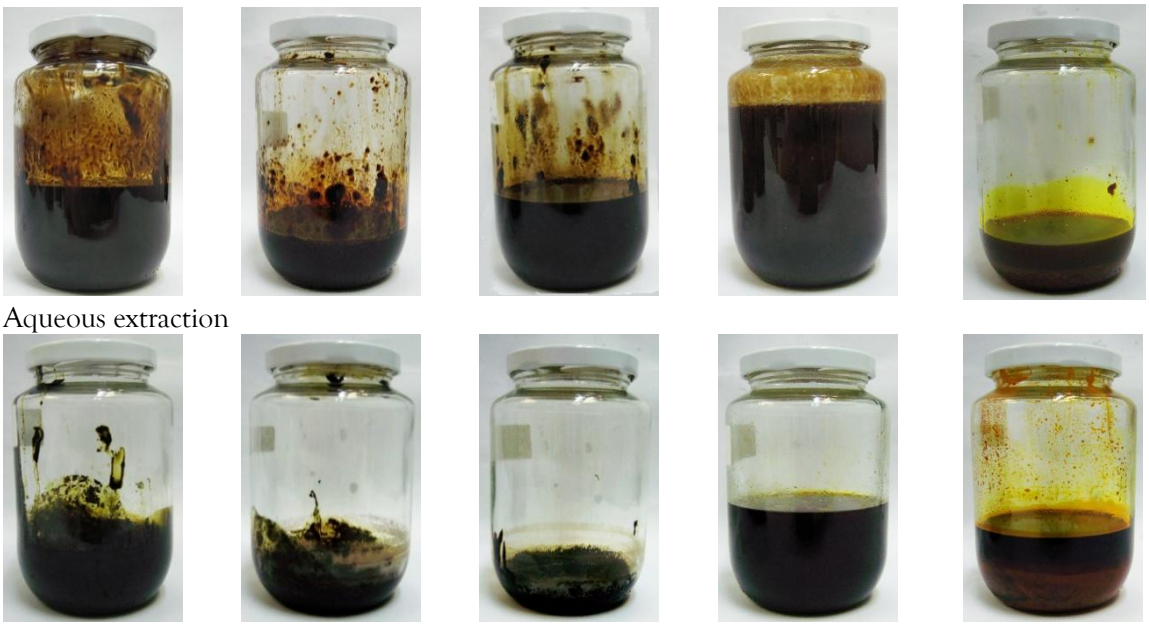


Fig. 2 Aqueous and Ethanol extracts

B. Chromatogram Analysis

The experimental results of HPTLC (System 1) and TLC (System 2) are presented in Fig. 3 and Fig. 4, using aqueous and ethanol extracts prepared for each crude sample. The samples were divided into three groups: (1) without development, (2) iodine vapor development, and (3) 10% sulfuric acid development. Each stationary phase was visually observed under UV light at 254 nm and 366 nm, allowing for the

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 20s, 2025
https://theaspd.com/index.php

detailed characterization of each crude sample on the stationary phase (extraction designation were shown in Table II).

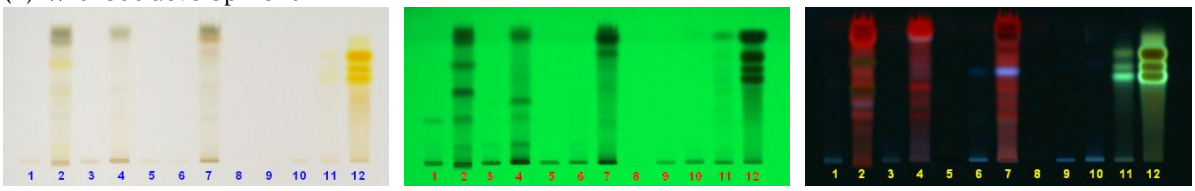
Observation of chromatogram bands revealed that crude extracts obtained using ethanol exhibited more intense bands than those extracted with water, indicating that ethanol could extract a wider variety of bioactive compounds. Additionally, band development using iodine vapor and 10% sulfuric acid spraying enhanced the visibility of color bands, as these reagents reacted with the crude extracts to produce sharper and clearer bands at equal extract concentrations.

Furthermore, the application of TLC and HPTLC techniques with iodine vapor and 10% sulfuric acid spraying facilitated the identification of Charantin in System 1, with a distinct band observed in sample number 8 on the stationary phase, corresponding to a rate of flow (R_f) value of 0.26. This confirmed the presence of Charantin in the aqueous extracts of Sapindus emarginatus and Curcuma longa, as they exhibited chromatogram bands at the same position with the same R_f value. In contrast, Charantin was detected in the ethanol extracts of all tested crude samples.

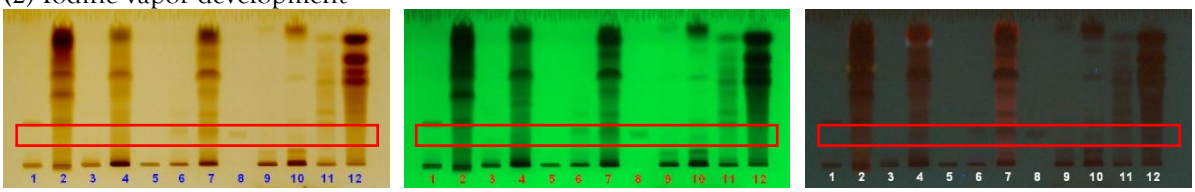
Although Rutin was not detected in System 1, it was identified in System 2 at an R_f value of 0.46, but only in both aqueous and ethanol extracts of Sapindus emarginatus. Developing TLC and HPTLC techniques using iodine vapor and 10% sulfuric acid spraying and adjusting the mobile phase systems improved the identification of specific compounds in the extracts. This enhancement allowed more precise visualization of chromatogram bands for previously indistinct compounds.

For instance, Charantin became more detectable in System 1 after development, though Rutin remained undetectable. The difference between System 1 and System 2, involving different polarity solvents, successfully facilitated the visualization of Rutin bands, highlighting the impact of solvent polarity on chromatographic separation.

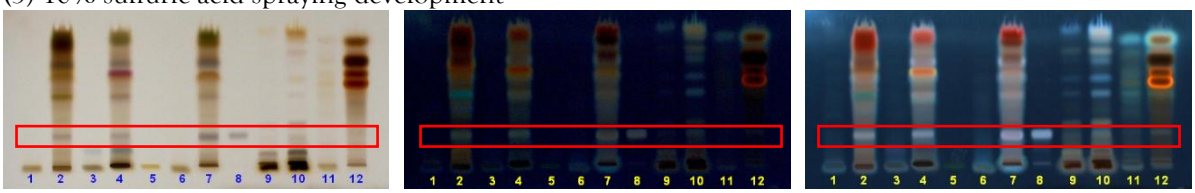
(1) Without development



(2) Iodine vapor development



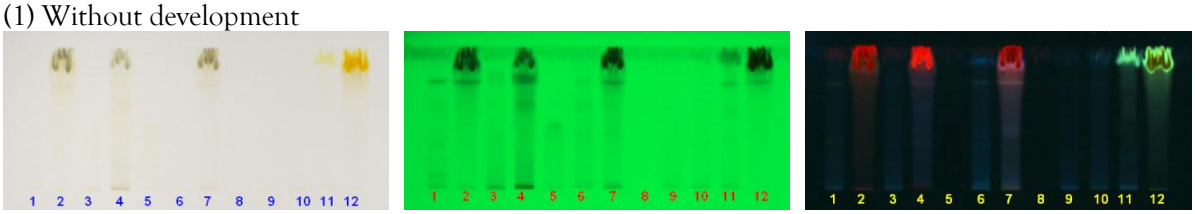
(3) 10% sulfuric acid spraying development



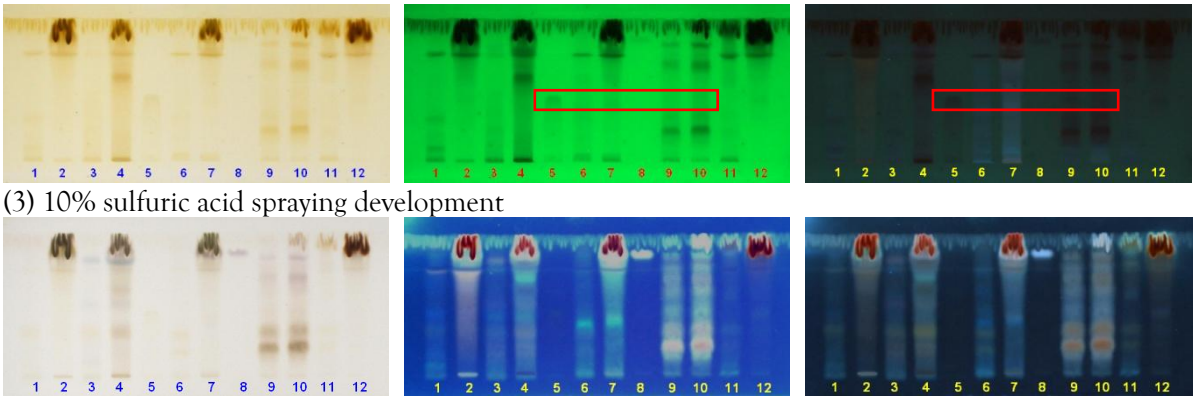
Visible 254 nm UV-Vis 366 nm UV-Vis

Fig. 3 HPTLC of each extraction (System 1)

(1) Without development



(2) Iodine vapor development



(3) 10% sulfuric acid spraying development

Visible 254 nm UV-Vis 366 nm UV-Vis

Fig. 4 TLC of each extraction (System 2)

TABLE II EXTRACTION DESIGNATION

Extraction designation	Sample No.	
	Aqueous extraction	Ethanol extraction
Cassia alata	1	2
Lawsonia inermis	3	4
Rhinacanthus nasutus	6	7
Sapindus emarginatus	9	10
Curcuma longa	11	12
Designation for standard marker		
Rutin	5	
Charantin	8	

C. Antibacterial Activity

1) Maximum growth rate

The results show that three bacteria strains reach their maximum growth rate in the 17-20 hours range, as shown in Table III.

TABLE III

MAXIMUM GROWTH RATE OF EACH BACTERIA STRAIN

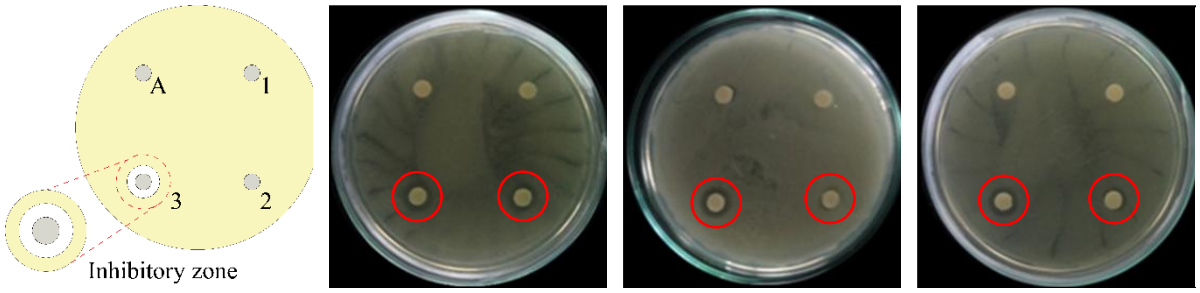
Bacteria	Maximum growth rate (hr.)
P. aeruginosa	16
S. aureus	20
S. epidermidis	18

2) Quantitative Analysis

Fig. 5 illustrates the inhibition of bacterial growth for each strain using McFarland Standard No. 0.5 at volumes of 0, 10, 20, and 30 µl. No bacterial growth inhibition was observed across all bacterial strains at 0 µl (A) and 10 µl (1). However, antibacterial activity began at 20 µl (2), showing an inhibitory zone of 7-7.5 mm. The most significant inhibition occurred at 30 µl (3), with an inhibitory zone of 8-9.5 mm, as detailed in Table IV. Consequently, the 30 µl volume of McFarland Standard No. 0.5 was selected as the standard control for evaluating the antibacterial activity of each crude extract, both in aqueous and ethanol forms.

TABLE IV INHIBITORY ZONE OF MCFARLAND STANDARD NO. 0.5

Bacteria	Inhibitory zone (mm.) of McFarland Standard No. 0.5			
	0 µl	10 µl	20 µl	30 µl
P. aeruginosa	-	-	7.50	8.00
S. aureus	-	-	7.00	8.50
S. epidermidis	-	-	7.00	9.50

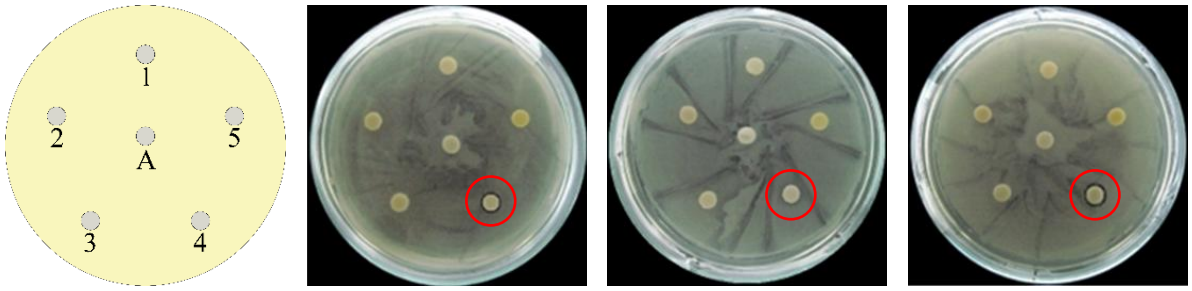


Extraction's disc layout P. aeruginosa S. aureus S. epidermidis

(A) means 20 µl Sterilized deionized water, (1), (2), and (3) mean 10, 20, and 30 µl of McFarland Standard No. 0.5

Fig. 5 Inhibitory zone of McFarland Standard No. 0.5

For aqueous extract, Fig. 6 demonstrates antibacterial activity by aqueous extracts of various crude samples for aqueous extract. Analysis of the extraction's disc layout from the inhibition images revealed that only the aqueous extract of Sapindus emarginatus exhibited antibacterial activity for all three bacterial strains. The inhibitory zones measured 8.21 mm for P. aeruginosa, 7.45 mm for S. aureus, and 6.89 mm for S. epidermidis, as shown in Table V. In contrast, other aqueous crude extracts showed no antibacterial activity against these bacterial strains.



Extraction's disc layout P. aeruginosa S. aureus S. epidermidis

Fig. 6 Inhibitory zone of the aqueous extracts

TABLE V

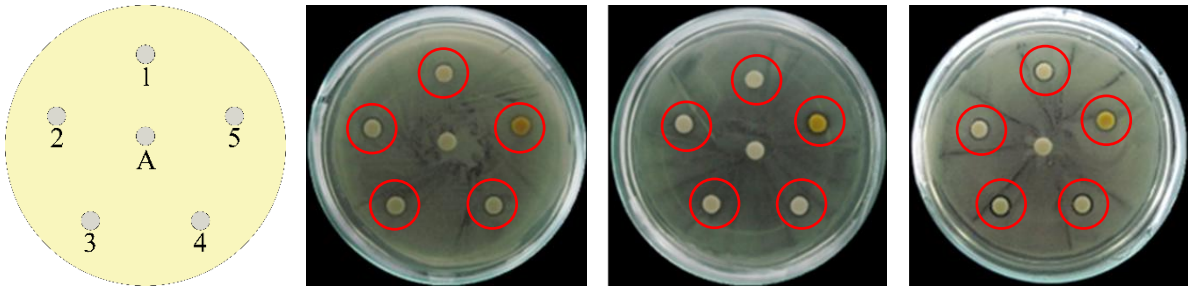
INHIBITORY ZONE OF EACH CRUDE AQUEOUS EXTRACT

Aqueous extracts		Bacteria		
		P. aeruginosa	**S. aureus**	**S. epidermidis**
Inhibitory zone (mm)	Cassia alata	-	-	-
	Lawsonia inermis	-	-	-
	Rhinacanthus nasutus	-	-	-
	Sapindus emarginatus	8.21	7.45	6.89
	Curcuma longa	-	-	-

For ethanol extract, Fig. 7 illustrates the antibacterial activity of ethanol extracts from various crude samples against different bacterial strains. The results indicated that all ethanol extracts are antibacterial against three skin pathogenic bacteria strains. Each extract exhibited comparable antibacterial efficacy, with the inhibitory zones detailed in Table VI. Notably, the ethanol extract of Rhinacanthus nasutus demonstrated the highest inhibition against P. aeruginosa and S. aureus, with inhibitory zones measuring 7.85 mm and 8.93 mm, respectively. In contrast, the ethanol extract of Lawsonia inermis had the most significant antibacterial activity against S. epidermidis, with an inhibitory zone of 8.48 mm.

All ethanol extracts exhibited antibacterial properties against all three skin pathogenic bacteria strains. However, only the extracts from Sapindus emarginatus demonstrated antibacterial activity in both

aqueous and ethanol forms. Although the ethanol extract of Sapindus emarginatus did not show the highest antibacterial efficacy compared to other ethanol extracts, it has antibacterial activity in both extraction methods, highlighting its broad-spectrum antimicrobial potential.



Extraction disc layout P. aeruginosa S. aureus S. epidermidis

Fig. 7 Inhibitory zone of the ethanol extracts

TABLE VI INHIBITORY ZONE OF EACH CRUDE ETHANOL EXTRACT

Ethanol extracts		Bacteria		
		P. aeruginosa	S. aureus	S. epidermidis
Inhibitory zone (mm)	Cassia alata	7.66	8.26	7.94
	Lawsonia inermis	7.68	7.21	8.48
	Rhinacanthus nasutus	7.85	8.93	8.03
	Sapindus emarginatus	7.74	8.76	7.94
	Curcuma longa	7.14	8.46	8.09

3) Minimum inhibitory concentration (MIC)

The results of the MIC test are presented in Table VII and align with the inhibitory zone test for each extract (Table V and Table VI). No antibacterial activity was observed for the aqueous extracts except for Sapindus emarginatus, which has antibacterial activity for all three skin pathogenic bacteria strains. The minimum concentrations required to inhibit the growth of P. aeruginosa, S. aureus, and S. epidermidis were $1x10^{-4}$, $1x10^{-2}$, and $1x10^{-2}$ mg/ml, respectively (as shown in Table VII).

All the ethanol extracts exhibited the ability to inhibit bacterial growth for all three strains. The most effective ethanol extracts were those from Rhinacanthus nasutus and Sapindus emarginatus. The minimum concentrations needed to inhibit P. aeruginosa, S. aureus, and S. epidermidis were $1x10^{-3}$, $1x10^{-4}$, and $1x10^{-3}$ mg/ml, respectively (as shown in Table VII).

TABLE VII

MINIMUM INHIBITORY CONCENTRATION OF EACH EXTRACT

Extraction		Bacteria		
		P. aeruginosa	S. aureus	S. epidermidis
MIC (mg/ml)	**Aqueous extracts**			
	Cassia alata	-	-	-
	Lawsonia inermis	-	-	-
	Rhinacanthus nasutus	-	-	-
	Sapindus emarginatus	$1x10^{-4}$	$1x10^{-2}$	$1x10^{-2}$
	Curcuma longa	-	-	-
	Ethanol extracts			
	Cassia alata	$1x10^{-1}$	$1x10^{-1}$	$1x10^{-1}$
	Lawsonia inermis	$1x10^{-2}$	$1x10^{-2}$	$1x10^{-2}$
	Rhinacanthus nasutus	$1x10^{-3}$	$1x10^{-4}$	$1x10^{-3}$
	Sapindus emarginatus	$1x10^{-3}$	$1x10^{-4}$	$1x10^{-3}$
	Curcuma longa	$1x10^{-3}$	$1x10^{-3}$	$1x10^{-3}$

IV. CONCLUSIONS

Based on the results obtained, the conclusions could be drawn:

• Developing techniques such as iodine vapor and 10% sulfuric acid spraying can enhance the visibility of the chromatogram of crude extracts on the stationary phase, making it easier to compare with standard substances.

• Bioactive compounds with antibacterial effects have been identified, including Rutin, in the crude aqueous and ethanol extracts of Sapindus emarginatus. Charantin was detected in all ethanol extracts of the medicinal plants and the aqueous extract of Sapindus emarginatus. The extracts containing these compounds showed antibacterial activity.

• Sapindus emarginatus was found to have antibacterial activity among the crude aqueous extracts, and all crude ethanol extracts showed antibacterial activity.

ACKNOWLEDGEMENT

This research was funded by King Mongkut's University of Technology North Bangkok. Contact no. KMUTNB-61-GOV-03-B-27.

REFERENCES

[1] C. Gupta and D. Prakash, "Medicinal plants in natural health care as phytopharmaceuticals," in Herbal Product Development, 1st ed., Palm Bay, FL: Apple Academic Press, pp. 1-36, 2020.

[2] R. Tiwari, S. Mishra, A. Chauhan, P. Gulati, and M. Dhobi, "The dawn till dusk of phytopharmaceuticals," Saudi Pharmaceutical Journal, vol. 32, no. 11, pp. 1-10, Oct. 2024.

[3] N. Nasim, I. S. Sandeep, and S. Mohanty, "Plant-derived natural products for drug discovery: current approaches and prospects," Nucleus, vol. 65, pp. 399–411, Oct. 2022.

[4] M. Phumthum, K. Srithi, A. Inta, A. Junsongduang, K. Tangjitman, W. Pongamornkul et al., "Ethnomedicinal plant diversity in Thailand," J. Ethnopharmacol., vol. 214, pp. 90–98, Mar. 2018.

[5] K. Maneenoon, C. Khuniad, Y. Teanuan, N. Saedan, S. Prom-in, N. Rukleng et al., "Ethnomedicinal plants used by traditional healers in Phatthalung Province, Peninsular Thailand," J. Ethnobiol. Ethnomed., vol. 11, no. 43, pp. 1-20, May 2015.

[6] S. O. Majekodunmi and A. A. Essien, "Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of Cassia alata for skin diseases," J. Coastal Life Med., vol. 2, no. 11, pp. 872–875, Jun. 2014.

[7] A. Mannan, M. J. Kawser, A. M. A. Ahmed, N. N. Isalm, S. M. M. Alam, M. A. E. K. Emon et al., "Assessment of antibacterial, thrombolytic, and cytotoxic potential of Cassia alata seed oil," Journal of Applied Pharmaceutical Science, vol. 01, no.09, pp. 56-59, Oct. 2012.

[8] K. F. Miwonouko, A. Dermane, B. Kombate, E. H. Gbekley, K. Anani, K. Metowogo et al., "Antioxidant and antimicrobial activities of Cassia alata (L.) Roxb. leaves," Int. J. Biol. Chem. Sci., vol. 18, no. 1, May 2024.

[9] O. Khantamat, N. Dukaew, J. Karinchai, T. Chewonarin, P. Pitchakarn, and P. Temviriyanukul, "Safety and bioactivity assessment of aqueous extract of Thai henna (Lawsonia inermis Linn.) leaf," J. Toxicol. Environ. Health, Part A, vol. 84, no. 7, pp. 298–312, Dec. 2020.

[10] V. Nagasinduja and S. Shahitha, "In-vitro antimicrobial activity of green synthesised silver nanoparticles of leaf extract of Rhinacanthus nasutus against bacterial food borne pathogens," J. Clin. Diagn. Res., vol. 16, no. 8, pp. 22–27, Aug. 2022.

[11] A. Ali, A. Arwa, A. Inas, P. Vidya, and F. Mazahar, "Phytochemical screening, GC-MS analysis, molecular docking study and evaluation of antioxidant and antimicrobial activity of Sapindus emarginatus seed kernel," Res. J. Pharm. Technol., vol. 15, no. 5, pp. 2117–2121, Feb. 2023.

[12] M. Górski, J. Niedźwiadek, and A. Magryś, "Antibacterial activity of curcumin – a natural phenylpropanoid dimer from the rhizomes of Curcuma longa L. and its synergy with antibiotics," Ann. Agric. Environ. Med., vol. 29, no. 3, pp. 394–400, May 2022.

[13] M. N. Colin, C. N. S. E. Claudiana, A. U. Kaffah, A. N. Hasanah, and S. Megantara, "Review on Cassia alata bioactive compounds: In silico, in vitro, and in vivo studies," Drug Des. Dev. Ther., vol. 18, pp. 4427–4447, Oct. 2024.

[14] R. K. Verma, P. Kumari, R. K. Maurya, V. Kumar, R. B. Verma, and R. K. Singh, "Medicinal properties of turmeric (Curcuma longa L.): A review," Int. J. Chem. Stud., vol. 6, no. 4, pp. 1354–1357, Jun. 2018.

[15] B. Jyotirmayee and G. Mahalik, "A review on selected pharmacological activities of Curcuma longa L.," Int. J. Food Prop., vol. 25, no. 1, pp. 1377–1398, May 2022.

[16] A. Moutawalli, F. Z. Benkhouili, A. Doukkali, H. Benzeid, and A. Zahidi, "The biological and pharmacologic actions of Lawsonia inermis L.," Phytomedicine Plus, vol. 3, no. 3, pp. 1-15, Aug. 2023.

[17] W. Yaowachai, P. Luecha, and W. T. Taratima, "In vitro callus induction and evaluation of antioxidant activity of Rhinacanthus nasutus (L.) Kurz," Biol. Methods Protoc., vol. 8, no. 1, pp. 1-10, Sep. 2023.

[18] G. C. J. Swarnavalli, S. Dinakaran, N. Raman, R. Jegadeesh, and C. Pereira, "Bio-inspired synthesis of monodispersed silver nanoparticles using Sapindus emarginatus pericarp extract – Study of antibacterial efficacy," J. Saudi Chem. Soc., vol. 21, no. 2, pp. 172–179, Feb. 2017

[19]