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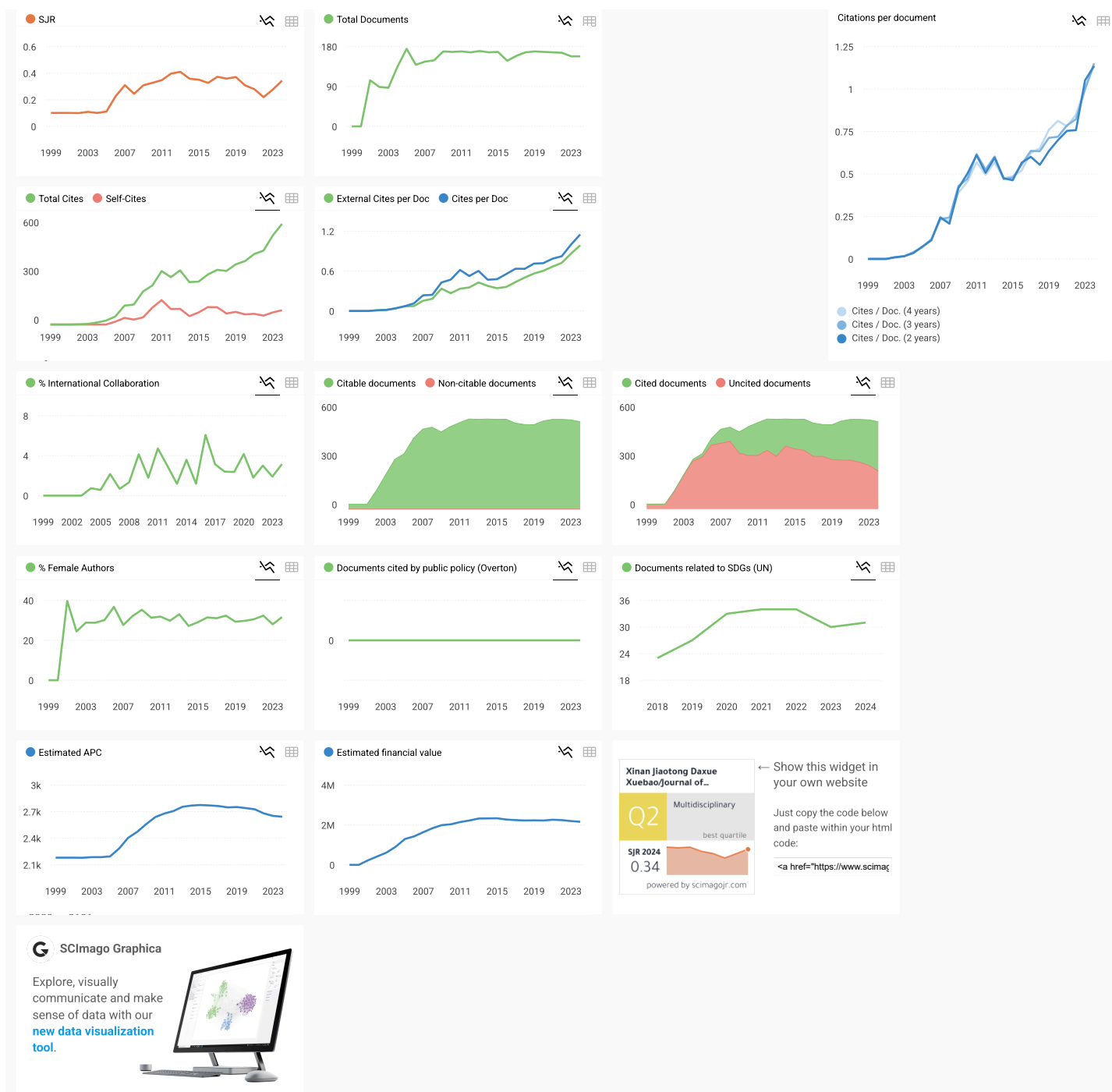
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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT PROPERTIES, AND ANTI-MRSA ACTIVITIES OF CLINACANTHUS NUTANS EXTRACT

垂穗草提取物的植物化学成分、抗氧化特性和抗耐甲氧西林金黄色葡萄球菌活性

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Abstract:

Clinacanthus nutans is recognized for its anticancer and antiviral properties. This study delineates a pioneering exploration of the antioxidant and anti-MRSA properties of extracts from *Clinacanthus nutans* (CN) leaves, predicated on their copious bioactive constituents, specifically total phenolic compounds (TPC) and total flavonoid compounds (TFC). Through the utilization of ethyl acetate extraction, phytochemical screening, DPPH, ABTS, FRAP, and antimicrobial assay techniques, the researchers exhibited that the unrefined CN extract displayed antibacterial efficacy against three variants of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 29213, MRSA 4738, and MRSA 20649). The experimental results of the phytochemical analysis revealed that the extract

contains tannins and flavonoids. The polyphenol compounds and flavonoids content of CN leaves extract in the ethyl acetate fraction had also been reported with higher flavonoids, 350.61 mg QE/g crude extract. In this study, the antioxidant activity of CN extracts in ethyl acetate solvent with DPPH, ABTS and FRAP was 53.88, 51.61 and 69.19 mg TE/g crude extract, respectively. The MIC and MBC values against *Staphylococcus aureus* ATTC 29213 were identified as 8 and 16 mg/ml, respectively. In addition, the CN crude extract demonstrated MIC and MBC values of 3.125 mg/ml and 6.25 mg/ml against *Staphylococcus aureus* (MRSA) DMST 4738 and *Staphylococcus aureus* (MRSA) DMST 20649, respectively. This approach facilitates the enhancement of antibacterial efficacy by diminishing the MIC and MBC of CN extracts via the recognition and use of particular bioactive compounds. New research findings enhance the current knowledge of antibacterial agents and support the development of cost-effective therapies, reducing reliance on imported drugs and health care costs.

Keywords: *Clinacanthus nutans*, phytochemical, antibacterial, Methicillin-resistant *Staphylococcus aureus* (MRSA), antioxidant

摘要 垂穗草因其抗癌和抗病毒特性而广受认可。本文概述了一项开创性的研究，旨在探究垂穗草（CN）叶提取物的抗氧化和抗耐甲氧西林金黄色葡萄球菌（MRSA）特性，该特性基于其丰富的生物活性成分，特别是总酚化合物（TPC）和总黄酮化合物（TFC）。研究人员通过乙酸乙酯提取、植物化学筛选、DPPH、ABTS、FRAP 和抗菌检测技术，发现未精制的 CN 提取物对三种金黄色葡萄球菌和耐甲氧西林金黄色葡萄球菌（MRSA）（ATCC 29213、MRSA 4738 和 MRSA 20649）均具有抗菌效果。植物化学分析实验结果表明，该提取物含有单宁和黄酮类化合物。据报道，CN 叶提取物在乙酸乙酯级分中的多酚化合物和黄酮类化合物含量较高，黄酮类化合物含量为 350.61 mg QE/g 粗提取物。在本研究中，在含有 DPPH、ABTS 和 FRAP 的乙酸乙酯溶剂中，CN 提取物的抗氧化活性分别为 53.88、51.61 和 69.19 mg TE/g 粗提取物。对金黄色葡萄球菌 ATCC 29213 的 MIC 和 MBC 值分别为 8 和 16 mg/ml。此外，CN 粗提取物对金黄色葡萄球菌（MRSA）DMST 4738 和金黄色葡萄球菌（MRSA）DMST 20649 的 MIC 和 MBC 值分别为 3.125 mg/ml 和 6.25 mg/ml。这种方法通过识别和利用特定的生物活性化合物，降低 CN 提取物的 MIC 和 MBC，从而增强抗菌功效。新的研究成果增强了人们对抗菌剂的现有认识，并支持开发经济有效的疗法，从而减少对进口药物的依赖和医疗保健成本。

关键词: 垂穗斜棘、植物化学物质、抗菌、耐甲氧西林金黄色葡萄球菌（MRSA）、抗氧化剂

I. INTRODUCTION

The global proliferation of bacterial infections is increasingly manifesting in more severe forms today. Moreover, the prevalence of irrational pharmaceutical consumption is on the rise. Bacteria are evolving both structural and genetic adaptations to counteract pharmacological interventions, which is a significant challenge in the realm of public health. As a result, there has been a growing interest in policies that endorse therapeutic strategies using phytomedicines or traditional Thai medicinal practices. For instance, the incidence of nosocomial infections has been steadily increasing. *Staphylococcus aureus* poses a considerable threat in the context of nosocomial infections, including pneumonia, surgical site infections, and sepsis [1]. Infections such as furuncles, boils, phlebitis, meningitis, endocarditis, and urinary tract infections all facilitate the

dissemination of *S. aureus* [2]. When the causative agent is identified as *S. aureus*, the mortality rate associated with nosocomial endocarditis surpasses that of urinary tract infections. An abscess represents a lesion caused by staphylococcal infection, characterized by a fibrinous wall encapsulating a central core comprising pus-laden microorganisms and leukocytes, enveloped by inflammatory tissue. Even in the most diminutive abscesses, the pathogens may disseminate via hematogenous routes. *S. aureus* tends to metastasize to specific anatomical sites, including the bones, joints, kidneys, and pulmonary tissue.

Many strains of *Staphylococcus aureus* are exhibiting increasing resistance to antimicrobial therapies, thereby presenting a significant public health risk, particularly exemplified by methicillin-resistant *Staphylococcus aureus* (MRSA). The organism may acquire genes that encode for enzymes such as β -lactamase, which

function to degrade antibacterial agents before their exertion of therapeutic effects. There is an urgent necessity to identify and develop novel antibacterial agents to counteract these challenges.

Clinacanthus nutans represents a widely recognized and promising source of antibacterial bioactive compounds [3]. This plant typically grows in conjunction with other arboreal species, reaching heights of approximately 1 to 3 meters. The stem exhibits a cylindrical shape with a smooth, green epidermis. Propagation can be successfully achieved through either cuttings or rhizome division. This species demonstrated robust growth across various soil types. Its geographical distribution includes regions within China, Vietnam, Indonesia, Malaysia, and Thailand. It is frequently encountered in mixed forest ecosystems throughout these countries or cultivated in residential gardens. The morphology of the leaves is characterized by a pear-shaped, oval, and narrow configuration with parallel margins. The foliage appears dark green and exhibits a smooth texture. The floral structure comprises red-orange petals that are fused into tubular formations, culminating in a bifurcated apex, consisting of both lower and upper lobes. The flowers are composed of two male anthers, whereas the female structure is glabrous, with a flowering period spanning from approximately October to January. Consequently, the extracts derived from *Clinacanthus nutans* are prevalent in antibacterial compounds and are commonly employed in traditional medicinal practices. Historically, these bioactive constituents have been used as primary pharmacologically active agents in traditional herbal remedies to alleviate human ailments. Moreover, ongoing antimicrobial investigations are being conducted on this particular class of natural products. The structural components have been identified within numerous categories of pure compounds that exhibit efficacy against viral, fungal, and bacterial pathogens.

The rich array of bioactive constituents, including flavonoids, betulin, lupeol, n-butanol, and glycolglycerolipids present in *Clinacanthus* species, has garnered significant attention from phytochemical researchers [4]. Numerous isolated compounds demonstrate an extensive spectrum of biological activities. For instance, both betulin and lupeol have been documented to possess anti-inflammatory and analgesic properties. Evidence has substantiated the presence of antiviral, antibacterial, and antimycobacterial activities across various studies. The mechanisms by which flavonoids derived from medicinal plants exert their antibacterial effects have been elucidated in multiple investigations. Owing to the structural

diversity within this class of phytochemicals, researchers have successfully identified a wide array of operative mechanisms for these compounds. The antimicrobial properties of the plant have been empirically validated. Nonetheless, the specific mechanism of action remains undocumented. Therefore, the objective of this research was to explore the phytochemical composition, antioxidant properties, and anti-MRSA efficacy of the extract. The findings from this research are anticipated to provide scientific validation for the development of prototypes aimed at combating drug resistance, thereby supporting the health policies set forth by the Ministry of Public Health. In particular, the application of medicinal plants in the management of bacterial infections is expected to mitigate the financial burden associated with the importation of antibacterial pharmaceuticals.

II. MATERIALS AND METHODS

A. Chemicals and Reagents

Hexane, ethanol, methanol, dichloromethane, ethyl acetate, NaCl, agar, Mueller-Hinton medium, 0.5 McFarland standard, CDC13, glutaraldehyde, acetone, and dimethyl sulfoxide were purchased from Carlo Erba (Italy). Vancomycin was purchased from Sigma-Aldrich (USA).

B. Plant Materials and Crude Herbal Extraction

Clinacanthus nutans was obtained from the province of Phetchabun, Thailand. Authentication of the plant material was performed by Dr. Surangrut Punsang from the Biology Program at Phetchabun Rajabhat University (PCRU). A voucher specimen (PCRU-CN-001) was placed in the custody of the Public Health Program at PCRU. The leaves underwent air-drying at ambient temperature and were subsequently stored until required for the extraction process. A quantity of one hundred grams of dried leaves underwent maceration for seven days in 500 mL of ethyl acetate. The resultant combined extracts were filtered through Whatman No. 1 filter paper. Subsequently, the filtrates were subjected to rotary evaporation, followed by freeze-drying. The purity of the extract was validated by thin-layer chromatography (TLC). The raw extract was dissolved in ethanol, and 5 μ L of the solution was administered onto TLC plates that were coated with silica gel G60 F254. An eluent of dichloromethane (95:5) was used. The visualization of the spot occurred under UV light at 254 nm to ascertain the purification process.

The constituents present in the *C. nutans* extract were determined via phytochemical screening, which revealed the existence of terpenoids, flavonoids, saponins, tannins, and cardiac glycosides. The execution of the phytochemical screening was based on methodologies previously documented [5].

C. Total Phenolic Content Analysis

The total phenolic content was determined using the Folin-Ciocalteu method. A standard calibration curve was constructed using various concentrations of gallic acid (GA) standard solutions at 10, 30, 50, 70, 100, 150, and 200 ppm. For each concentration, 5 mL of 10% Folin-Ciocalteu's reagent was added to a 15 mL test tube, followed by 1 mL of the gallic acid standard solution. The mixture was incubated in the dark at room temperature for 5 minutes. Afterward, 4 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the mixture was left in the dark at room temperature for 15 minutes, resulting in a total volume of 10 mL. This procedure was performed in triplicate. The absorbance of each concentration was measured at 765 nm using an ultraviolet-visible (UV-VIS) spectrophotometer, and a standard curve was plotted with the concentration (ppm) on the x-axis and the absorbance on the y-axis. The total phenolic content of the extracts was analyzed in the same manner as the standard solutions. The absorbance values of the samples were compared to the standard curve, and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GA/g extract).

D. Total Flavonoid Content Analysis

The total flavonoid content was determined using the Aluminum chloride method. A standard calibration curve was constructed using quercetin standard solutions at concentrations of 50, 100, 200, 300, 400, 500, and 600 ppm. For each concentration, 0.6 mL of the standard solution was added to a 15 mL test tube, followed by the addition of 2.4 mL of distilled water. Next, 0.3 mL of 5% sodium nitrite (NaNO_2) solution was added, and the mixture was shaken and left to stand for 5 minutes. Afterward, 0.3 mL of 10% aluminum chloride (AlCl_3) solution was added, shaken, and left to stand for another 5 minutes. Then, 1 mL of 1 M sodium hydroxide (NaOH) solution and 1.4 mL of distilled water were added to bring the total volume to 6 mL. The mixture was left in the dark at room temperature for 15 minutes, and the absorbance was measured at 415 nm using an ultraviolet-visible (UV-VIS) spectrophotometer. The standard curve was plotted with the

concentration (ppm) on the x-axis and the absorbance on the y-axis.

The total flavonoid content in the extract samples was analyzed in the same manner as the standard solutions. The absorbance values of the samples were compared to the standard curve, and the total flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

E. DPPH Radical Scavenging Activity Analysis

The DPPH radical scavenging activity was determined using a modified method with Trolox as the standard solution at concentrations of 10, 20, 30, 40, 50, 60, and 70 ppm. For the analysis, 0.5 mL of either the standard or the sample solution was added to a 15 mL test tube, followed by the addition of 4.5 mL of 200 mM DPPH solution. The mixture was left to stand in the dark at room temperature for 30 minutes, resulting in a total volume of 5 mL. This procedure was performed in triplicate. The absorbance was measured at 517 nm using an ultraviolet-visible (UV-VIS) spectrophotometer. The measured absorbance (A) was used to calculate the percentage of radical scavenging activity (% inhibition) according to Equation 1. A standard curve was then constructed with percent inhibition on the y-axis and the Trolox concentration on the x-axis. The absorbance of the samples was also measured, and the percent inhibition was calculated using the same equation. The values obtained were compared to the standard curve, and the DPPH radical scavenging activity was expressed as milligrams of Trolox equivalents per gram of extract (mg TE/g extract).

$$\% \text{ radical scavenging activity} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100,$$

where A_{control} refers to the absorbance value of the ABTS or DPPH solution, and

A_{sample} refers to the absorbance value of the sample solution or the standard solution.

F. ABTS Radical Scavenging Activity Analysis

The ABTS radical scavenging activity was determined using a modified method with Trolox as the standard solution at concentrations of 10, 20, 30, 40, 50, 60, and 70 ppm. For the analysis, 0.5 mL of either the standard or the sample solution was added to a 15 mL test tube, followed by 4.5 mL of 200 mM ABTS solution. The mixture was allowed to stand at room temperature for 7

minutes, resulting in a total volume of 5 mL. This procedure was performed in triplicate. The absorbance was measured at 734 nm using an ultraviolet-visible (UV-VIS) spectrophotometer. The measured absorbance (A) was used to calculate the percentage of radical scavenging activity (% inhibition) according to Equation 1. A standard curve was then constructed on the y-axis and the Trolox concentration on the x-axis. The absorbance of the samples was also measured, and the percent inhibition was calculated using the same equation. The values obtained were compared to the standard curve, and the ABTS radical scavenging activity was expressed as milligrams of Trolox equivalents per gram of extract (mg TE/g extract).

G. FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP activity was determined using a modified method. A standard curve was constructed using Trolox as the standard solution at concentrations of 25, 50, 100, 150, 200, 250, and 300 ppm. For each concentration, 1 mL of distilled water was added to a 15 mL test tube, followed by 0.1 mL of the Trolox standard solution and 3 mL of FRAP reagent. The mixture was incubated at 37°C for 15 minutes, resulting in a total volume of 4.1 mL. This procedure was performed in triplicate. The absorbance was measured at 593 nm using an ultraviolet-visible (UV-VIS) spectrophotometer. A standard curve was then plotted with concentration (ppm) on the x-axis and the absorbance on the y-axis to calculate the reducing power of FRAP. The FRAP activity of the extract samples was analyzed in the same manner as the standard solutions. The absorbance values of the samples were compared to the standard curve, and the FRAP activity was expressed as milligrams of Trolox equivalents per gram of extract (mg TE/g extract).

H. Disc Diffusion

The bacterial strains employed in the current study were sourced from the Department of Medical Sciences Thailand (DMST). These strains comprised *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* (MRSA) DMST 20649, and *Staphylococcus aureus* (MRSA) DMST 4738. The antibacterial properties of the Ethyl acetate crude extract obtained from *Clinacanthus nutans* (*C. nutans*) were evaluated using the disk diffusion method. The process of preparing bacterial suspensions involved introducing a single loopful of a pure colony into Mueller-Hinton Broth (MHB), followed by an overnight incubation period. Subsequently, the cultures were

diluted with 0.85% NaCl. These cell suspensions were standardized to a turbidity equal to a 0.5 McFarland standard, which corresponds to around 1×10^8 CFU/mL. These suspensions were then used to inoculate the Mueller-Hinton Agar (MHA) plates by swab testing the entire surface of the agar. Circular filter paper disks (Whatman No. 1, 6 mm diameter) were saturated with the *C. nutans* crude extract at concentrations of 25, 50, and 75 µg/disc and positioned on the previously inoculated agar plates. Following a 24-hour period of incubation at 37°C, the efficacy of the antibacterial activity was determined by measuring the diameter of the zones of inhibition surrounding the disks. Vancomycin and DMSO served as the positive and negative controls, respectively [6].

I. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The researchers utilized an adapted broth microdilution technique following the protocols established by the Clinical and Laboratory Standards Institute [7] to assess the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract. The extract was dissolved in DMSO and underwent serial dilution in Mueller-Hinton Broth (MHB) within a 96-well flat-bottom microtiter plate (Corning Life Sciences, USA). A bacterial suspension in MHB was prepared using an overnight broth culture, resulting in a final bacterial cell concentration of 5×10^5 CFU/mL [8]. The concentration range of the CN extract ranged from 0.25–512 mg/mL [9].

The positive and negative controls employed were vancomycin and DMSO, respectively. MIC was determined as the minimum concentration of the extract that displayed no visible microbial growth following a 24-h incubation at 37°C. For MBC assessment, 20 µL of broth from wells without any growth was subcultured on Mueller-Hinton Agar (MHA) plates and incubated at 37°C for 24 hours. MBC was identified as the lowest extract concentration that did not yield observable bacterial growth.

All experiments were repeated independently three times.

III. RESULTS

A. Phytochemical Screening

The outcome revealed that the CN extract unveiled tannins and flavonoid compound exposure (Table 1).

Table 1
Phytochemical screening of the ethyl acetate extract from *C. nutans* leaves (compiled by the authors)

Compounds	CN extract
Tannins	+
Terpenoids	-
Cardiac glycoside	-
Flavonoids	+
Saponins	-

Note: +: Presence and -: Absence of metabolites in the extract

A method known as Thin Layer Chromatography (TLC) was applied for studying the ethyl acetate extract. The outcomes of the extraction of the active compounds from *C. nutans* leaves displayed a dark green viscous texture post-maceration using TLC to validate the purity of the extract.



Figure 1. TLC of the CN extract was detected by UV light at 254 nm (developed by the authors)

Table 2.
Total polyphenol content and flavonoids of the *Clinacanthus nutans* extract (compiled by the authors)

Specific name	Part used	Solvent	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Family: Acanthaceae	leaves	ethyl acetate	8.9	54.955±0.08	350.61±0.71

C. DPPH, ABTS and FRAP

According to [12], the antioxidant assays of the CN extract with DPPH assay. The stable organic nitrogen radical 1,1-diphenyl-1-picrylhydrazyl (DPPH) was used in the DPPH assay, a decolorization method that turns yellow after changing from purple.

Table 3.
Antioxidant capacities of the crude extract from *Clinacanthus nutans* leaves in the ethyl acetate fraction (compiled by the authors)

Crude extract	Antioxidant activity		
	DPPH scavenging (mg TE/g)	TEAC values of ABTS (mg TE/g)	FRAP value (mg TE/g)
<i>Clinacanthus nutans</i>	53.88±0.50	51.61±0.75	69.19±0.05

In this study, the antioxidant activity of the CN extracts in the ethyl acetate solvent with DPPH,

The dark green viscous substance was dissolved in ethanol and subjected to TLC on aluminum plates coated with silica gel G60 F254, as depicted in Figure 1.

A mixture of Dichloromethane: Methanol (95:5) was employed as the eluent.

B. Total Phenolic and Total Flavonoid Content Analysis

Table 2 presents the yields of crude extracts, polyphenol compounds and flavonoids content of CN leaves extract in the ethyl acetate fraction, which had also been reported with higher flavonoids, 350.61 mg QE/g crude extract and the same resulted of reported the ethyl acetate fraction has a higher concentration of total phenol content (1082.05 mg GAE/g fraction) [10]. It was found that several phenolic compounds were identified and quantified to have high contents in ethyl acetate extracts of *C. nutans* by LC-MS analysis (sinapic acid, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, coumalic acid and quercetin hydrate) [11].

ABTS and FRAP was 53.88, 51.61 and 69.19 mg TE/g crude extract, respectively. The DPPH activity of the CN extracts was highest in the ethyl acetate and dichloromethane extracts. Numerous publications have demonstrated the exceptional antioxidant capability of solvent extracts from *Clinacanthus nutans*.

D. Disc Diffusion and MIC/MBC

According to the antibacterial activity, at 25, 50, and 75 µg, the CN crude extract inhibited the growth of *Staphylococcus aureus* ATTC 29213, *Staphylococcus aureus* (MRSA) DMST 20649, and *Staphylococcus aureus* (MRSA) DMST 4738, respectively, in a disc diffusion assay (Table 4). On the other hand, vancomycin was used as a positive control.

Table 4
Antibacterial activity of the CN crude extract using Disc diffusion (compiled by the authors)

Microorganism	Diameter of inhibitions zone (mm)			Vancomycin (µg)
	25	CN (µg/disc) 50	75	
<i>Staphylococcus aureus</i> 29213	12 ± 0.2	20 ± 0.6	25 ± 0.2	37 ± 0.5
<i>Staphylococcus aureus</i> (MRSA) 4738	10 ± 0.5	23 ± 0.6	25 ± 0.3	24 ± 0.7
<i>Staphylococcus aureus</i> (MRSA) 20649	10 ± 0.1	23 ± 0.2	26 ± 0.5	28 ± 0.7
Mean data ± SD (n=3)				

The minimal inhibition concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of the CN crude extract were determined using the microdilution method and are presented in Table 5. The MIC and MBC values against *Staphylococcus aureus* ATTC 29213 were identified as 8 and 16 mg/ml, respectively.

Furthermore, the CN crude extract demonstrated MIC and MBC values of 3.125 mg/ml and 6.25 mg/ml against *Staphylococcus aureus* (MRSA) DMST 4738 and *Staphylococcus aureus* (MRSA) DMST 20649, respectively. Each trial was conducted in triplicate.

Table 5.
The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the CN crude extract against MRSA compared to vancomycin (compiled by the authors)

Microorganisms	<i>Clinacanthus nutans</i>		Vancomycin	
	MIC (mg /ml)	MBC (mg /ml)	MIC (µg/ml)	MBC (µg/ml)
<i>Staphylococcus aureus</i> 29213	8	16	4	8
<i>Staphylococcus aureus</i> (MRSA) 4738	3.125	6.25	4	8
<i>Staphylococcus aureus</i> (MRSA) 20649	3.125	6.25	4	8

IV. DISCUSSION

This study investigated the antibacterial activity of *Clinacanthus nutans* (*C. nutans*) extract against *Staphylococcus aureus* (*S. aureus*), a Gram-positive pathogen known for its role in various skin and soft tissue infections, as well as its growing resistance to multiple antibiotics. Our findings demonstrate that the crude extract of *C. nutans* exhibits inhibitory effects on *S. aureus*, as evidenced by the clear zone of inhibition observed in the disc diffusion assay and the determined minimum inhibitory concentration (MIC) values.

The antibacterial activity of *C. nutans* observed in this study is consistent with previous reports suggesting its broad-spectrum antimicrobial potential [3], [13]. Several phytochemical constituents present in *C. nutans*, such as flavonoids, glycosides, and phenolic compounds, are known to possess antimicrobial properties. These bioactive compounds likely disrupt bacterial cell walls or interfere with the metabolic pathways critical for bacterial survival.

Compared to standard antibiotics, the zone of inhibition produced by the *C. nutans* extract was relatively smaller, suggesting that while the extract possesses antibacterial activity, it may be less potent than conventional antibiotics. However, the use of plant-derived compounds offers several advantages, including lower toxicity, reduced side effects, and a decreased likelihood of inducing antibiotic resistance.

Phytochemical screening in this research identified the presence of flavonoids, saponins, cardiac glycosides, tannins, and triterpenoids in the CN crude extracts, with tannins and flavonoids being the prominent constituents. Tannins, or tannic acid, are compounds from phenolic acids classified under the polyphenol category. Polyphenols, including tannins, can bind readily to proteins, cellulose, starch, and minerals, rendering tannins more insoluble and resilient to decomposition compared to other compounds within the same category.

This study evaluated the antioxidant and antibacterial activities of the *Clinacanthus nutans* (CN) crude extract using ethyl acetate as the solvent. The extract demonstrated strong antioxidant capacity, as assessed by three standard assays. The DPPH, ABTS, and FRAP results were 53.88, 51.61, and 69.19 mg TE/g of the crude extract, respectively. These findings indicate the presence of significant free radical scavenging and reducing power, attributed to the phytochemical constituents of CN, particularly flavonoids and phenolic compounds, which are well known for their electron-donating properties and antioxidant efficacy [14-17].

In terms of antibacterial activity, the CN extract was tested against *Staphylococcus aureus* ATCC 29213 and two methicillin-resistant strains (*S. aureus* (MRSA) DMST 4738 and DMST 20649) using the broth microdilution method. The extract exhibited notable antibacterial effects, with

minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 8 mg/mL and 16 mg/mL against *S. aureus* ATCC 29213. Remarkably, stronger antibacterial activity was observed against the MRSA strains, with MIC and MBC values of 3.125 mg/mL and 6.25 mg/mL, respectively. These results suggest that the CN extract may exert enhanced efficacy against resistant strains, potentially due to different mechanisms of bacterial susceptibility or cell wall structure.

The observed antibacterial activity is consistent with previous reports [18], which highlighted the antimicrobial potential of CN extracts against gram-positive and Gram-negative bacteria.

The presence of active secondary metabolites such as terpenoids, saponins, and alkaloids may contribute to cell wall disruption, protein synthesis inhibition, or interference with DNA replication in bacteria.

The dual biological activity of the CN extract offers promising therapeutic implications. In infectious conditions, oxidative stress often intensifies tissue inflammation and damage. Therefore, an agent with both antioxidant and antimicrobial properties could simultaneously accelerate healing and reduce the microbial load. This is especially relevant in chronic wound environments, where bacterial colonization and oxidative imbalance co-exist.

While these results are promising, the relatively high MIC/MBC values compared with standard antibiotics suggest that the crude extract may benefit from further purification to enhance potency. Additionally, variability in the phytochemical content due to the extraction solvent, plant maturity, and geographical factors should be considered when interpreting the biological activities. Ethyl acetate was selected as the extraction solvent in this study because of its intermediate polarity, which is effective in isolating flavonoids and phenolic acids that contribute significantly to the observed bioactivities.

V. CONCLUSION

The ethyl acetate crude extract of *Clinacanthus nutans* exhibited strong antioxidant activity and moderate to potent antibacterial activity, particularly against MRSA strains. These findings support its potential as a natural therapeutic agent for managing bacterial infections complicated by oxidative stress. Further research is warranted to explore its active constituents and clinical applicability.

A. Theoretical Contribution

This study enriches the phytochemical and pharmacological knowledge base of *Clinacanthus nutans* by providing the first quantitative description of the total phenolics and flavonoids in an ethyl-acetate leaf extract. By integrating the compositional analysis with the results from DPPH, ABTS, FRAP, disk-diffusion and broth microdilution assays, the work demonstrates a coherent link between high polyphenol content and dual antioxidant–anti-MRSA functionality. In methodological terms, it exemplifies a streamlined laboratory workflow—phytochemical profiling followed by parallel antioxidant and antimicrobial evaluation—that can be adopted as a template for the rapid screening of botanicals targeting drug-resistant pathogens. Beyond these empirical advances, the findings provide a conceptual basis for combination therapy: the extract’s ability to lower MIC and MBC values against clinical MRSA isolates positions plant-derived polyphenols as potential adjuvants capable of restoring or enhancing the effectiveness of conventional antibiotics.

B. Practical Significance

Because the extract displays both pronounced radical-scavenging capacity and measurable activity against methicillin-resistant *Staphylococcus aureus*, it offers tangible opportunities for product development in several applied domains. In pharmaceutical research, the results justify the progression to topical or oral formulations aimed at cutaneous and wound infections where oxidative stress and bacterial colonization co-exist. Healthcare systems in Southeast Asia could benefit economically by substituting or supplementing expensive imported antibiotics with locally sourced phytotherapeutics, thereby strengthening national policies that encourage traditional medicinal resources. The concurrent antioxidant effect supports cosmeceutical strategies focused on skin barrier protection and anti-acne interventions, while the prospect of adjuvant use suggests a route to lower antibiotic dosages, reduce adverse reactions, and slow the emergence of resistance.

C. Future Research Directions

Subsequent investigations should isolate and structurally characterize the individual metabolites responsible for the observed bioactivities, followed by mechanistic studies that track the transcriptional and phenotypic responses of MRSA to sub-inhibitory concentrations of these molecules. In vivo validation in wound or burn models is required to correlate microbial load

reduction with redox modulation in the tissue, accompanied by full toxicological and pharmacokinetic profiling. Formulation science should explore the nanocarriers and hydrogel matrices designed to stabilize polyphenols and optimize transdermal delivery. Synergy testing with frontline antibiotics will clarify the extract's potential to resensitize resistant strains, while agronomic research on cultivation conditions and post-harvest handling should establish standards that guarantee consistent phenolic and flavonoid yields for industrial scale-up.

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DECLARATIONS

Author Contributions

Conceptualization: Kamol Yusook (K.Y.); methodology, Kamol Yusook (K.Y.), and Tanawan Sukkasem (T.S.); software, Tanawan Sukkasem (T.S.); validation, Kamol Yusook (K.Y.), and Tanawan Sukkasem (T.S.); formal analysis, Tanawan Sukkasem (T.S.); investigation, Kamol Yusook (K.Y.), and Tanawan Sukkasem (T.S.); resources, Kamol Yusook (K.Y.); data curation, Kamol Yusook (K.Y.); writing—original draft preparation, all authors contributed equally; writing—review and editing, Kamol Yusook (K.Y.); visualization, Tanawan Sukkasem (T.S.); supervision, Kamol Yusook (K.Y.); project administration, Kamol Yusook (K.Y.). All authors have read and agreed to the published version of the manuscript.

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Ethical Compliance

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

Competing Interests

The authors confirm that no competing interests exist.

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