

Antibacterial Effect of Ethanol Extracts of *Murraya paniculata* Leaves, *Smallanthus sonchifolius* Leaves, *Apis trigona* Honey, and their Combination Against *Staphylococcus epidermidis*

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Abstract: Several studies have reported that combining plant extracts may enhance their efficacy against specific bacterial infections. This study aimed to analyze the antibacterial interaction of ethanol extracts of *Murraya paniculata* leaves, *Smallanthus sonchifolius* leaves, *Apis trigona* honey, and their combinations against *Staphylococcus epidermidis* ATCC 12228, as each component has shown individual antibacterial activity against this bacterium. The antibacterial interactions of the test materials, both individually and in combination, were evaluated using the agar diffusion method with clindamycin phosphate as the standard antibiotic. The minimum inhibitory concentration (MIC) of the most potent extract was determined through the microdilution method according to the *Clinical and Laboratory Standards Institute* (CLSI) guidelines, while the minimum bactericidal concentration (MBC) was assessed via subculture on solid media. Among all tested substances, the *S. sonchifolius* leaf extract exhibited the highest antibacterial activity, with similar MIC and MBC values ranging from 15.625 to 31.25 mg/mL. Interaction tests revealed a significant difference, showing that the combination of all three agents had an antagonistic effect, whereas the combination of both leaf extracts produced a synergistic antibacterial effect. However, the inhibitory effect of the combination was not greater than that of the yacon extract alone. In conclusion, *S. sonchifolius* leaf extract demonstrates strong potential as a single antibacterial agent against *S. epidermidis*.

Keywords: *Murraya paniculata*, *Smallanthus sonchifolius*, *Apis trigona*, *Staphylococcus epidermidis*, synergistic, antagonistic

Abstrak: Beberapa penelitian menunjukkan bahwa kombinasi ekstrak tumbuhan dapat meningkatkan efisiensinya melawan infeksi bakteri tertentu. Untuk itu, penelitian ini bertujuan menganalisis interaksi antibakteri dari ekstrak etanol daun kemuning, daun yacon, madu *Apis trigona*, dan kombinasinya terhadap *Staphylococcus epidermidis* ATCC 12228 yang diketahui bahwa masing-masing ekstrak dan madu tersebut memiliki daya antibakteri terhadap *S. epidermidis*. Efek interaksi antibakteri dari masing-masing bahan uji, baik secara tunggal maupun kombinasi, dievaluasi dengan metode difusi agar menggunakan klindamisin fosfat sebagai antibiotik standar. Konsentrasi hambat minimum (KHM) dari ekstrak paling potensial ditentukan dengan uji mikrodilusi sesuai dengan pedoman *Clinical and Laboratory Standards Institute* (CLSI). Nilai konsentrasi bunuh minimum (KBM) ditentukan dengan subkultur pada permukaan media padat. Di antara semua bahan uji, ekstrak daun yacon menunjukkan aktivitas penghambatan tertinggi dengan nilai KHM dan KBM yang serupa, yaitu 15,625–31,25 mg/mL. Hasil uji interaksi menunjukkan perbedaan signifikan, di mana kombinasi ketiga bahan uji bersifat antagonis, sedangkan kombinasi kedua ekstrak daun menghasilkan efek antibakteri yang bersifat sinergis. Meskipun demikian, daya hambat yang dihasilkan oleh kombinasi tersebut tidak sebesar daya hambat ekstrak yacon tunggal. Kesimpulannya, ekstrak daun yacon berpotensi kuat sebagai antibakteri tunggal terhadap *S. epidermidis*.

Kata kunci: kemuning, yacon, *Apis trigona*, *Staphylococcus epidermidis*, sinergis, antagonis

INTRODUCTION

Staphylococcus epidermidis is the most commonly isolated *Staphylococcus* species from the human epithelial surface, predominantly found in the axillary region, scalp, and nasal cavity (Severn & Horswill 2023). This bacterium is opportunistic and can infect individuals with weakened immune systems, leading to infections such as bacteremia. Currently, antibiotic-resistant *S. epidermidis* has become a major cause of clinical infections in hospitals. Considering the increasing antibiotic resistance among microorganisms, research aimed at discovering new antimicrobial agents is critically important. Data show that resistance of *S. epidermidis* to methicillin reaches 75–90%, a rate comparable to that of *S. aureus* infections (Lax & Gilbert 2015). The emergence and rapid spread of multidrug-resistant (MDR) bacteria have become a serious global public health concern (Pang *et al.* 2019). Excessive and inappropriate antibiotic use can promote bacterial tolerance, reducing the efficacy of antibiotics or rendering them ineffective, often through mechanisms such as antibiotic target modification (Baym 2016). Therefore, the search for novel antimicrobial compounds to combat infections caused by resistant bacteria is an urgent priority (Siddique *et al.* 2021).

Natural antimicrobial agents derived from medicinal plants are widely believed to contain phytochemical compounds with the potential to serve as safe and effective alternatives in disease treatment. Drug products from higher plants may offer candidates with distinct mechanisms of action to combat infections (Siddique *et al.* 2021; Keita *et al.* 2022). Combination therapy has emerged as a promising strategy in the fight against infectious diseases, particularly in the case of polymicrobial infections and those caused by resistant microorganisms. Such therapy can provide broader spectrum coverage and enhance treatment efficacy in patients with severe infections (Basavegowda & Baek 2022). Several studies have shown that combining plant extracts may enhance their efficiency against certain bacterial infections (Basavegowda & Baek 2022). This study aims to explore the antibacterial potential of a combination of *Murraya paniculata* leaves, *Smallanthus sonchifolius* leaves, and *Apis trigona* honey, each of which is known to contain phytochemical compounds with bactericidal activity against Gram-positive bacteria (Basavegowda & Baek 2022; Hendry *et al.* 2009; LaPlante 2007; Gunardi 2007). In this research, we further investigate the antibacterial effectiveness of these plant extracts in combination with honey against *S. epidermidis*, to determine and analyze whether there is a significant difference in antibacterial activity between the individual extracts and their combination.

MATERIALS AND METHOD

Materials

The plant materials were collected from the Manoko Plantation, West Java, Indonesia, and *Apis trigona* honey was obtained from the Ciburial Honey Bee Cultivation Center. All samples were authenticated by taxonomists from the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia. The identification results confirmed the samples as *Murraya paniculata* (L.) Jack, *Smallanthus sonchifolius* (Poepp.) H. Rob (No. 531/HB/02/2017), and *Apis trigona* (No. 557/HB/02/2018).

The bacterial strain used in this study was *Staphylococcus epidermidis* ATCC 12228. The culture media used included Tryptic Soy Agar (TSA; Oxoid), Mueller-Hinton Broth (MHB; Oxoid), and Mueller-Hinton Agar (MHA; Oxoid). The chemical reagents used were dimethyl sulfoxide (DMSO; Merck, Germany), Whatman No. 1 filter paper, Mayer's reagent (Sigma-Aldrich), Wagner's reagent (Sigma-Aldrich), sodium hydroxide (Sigma-Aldrich), chloroform (Sigma-Aldrich), ferric chloride (Sigma-Aldrich), and clindamycin phosphate (Fisher Bioreagent).

Extraction of *M. paniculata* and *S. sonchifolius*

Powdered *M. paniculata* leaves (500 g) and *S. sonchifolius* leaves (500 g) were macerated in 1 L of 70% ethanol for three days. After the maceration process, the resulting filtrate was filtered using Whatman No. 1 filter paper. The filtered extract was then evaporated using a rotary evaporator at 55 °C to obtain a concentrated extract. The concentrated extracts and honey were stored in sterile screw-cap bottles at 20 °C and were dissolved in dimethyl sulfoxide (DMSO; Merck, Germany) prior to use.

Phytochemical Screening

The secondary metabolites-alkaloids, flavonoids, saponins, steroids, and tannins present in *Apis trigona* honey and in the leaf extracts of *M. paniculata* and *S. sonchifolius* were identified using qualitative methods (Amina *et al.* 2013; Sajjad *et al.* 2015). Alkaloid detection was performed by mixing 2 mL of the extract with 2 mL of 10% hydrochloric acid, followed by the addition of a few drops of Mayer's or Wagner's reagent to 1 mL of the filtrate. The formation of a cream-colored precipitate upon the addition of Mayer's reagent or a reddish-brown precipitate after Wagner's reagent indicated the presence of alkaloids. For flavonoid detection, 3 mL of the filtrate was made alkaline using sodium hydroxide (NaOH), and the appearance of a yellow coloration indicated the presence of flavonoids. Saponins were detected by adding 5 mL of water to 2 g of the test sample, followed by vigorous shaking and standing for several minutes. The formation of stable froth indicated the presence of saponins. Steroids were identified by dissolving 5 g of the

Table 1. Test material combinations

Test Material	Combination		
	<i>M. paniculata</i>	<i>S. sonchifolius</i>	<i>A. trigona</i> honey
<i>M. paniculata</i>	-	1:1	1:1
<i>S. sonchifolius</i>	1:1	-	1:1
<i>A. trigona</i> honey	1:1	1:1	-

powdered sample in 5 mL of chloroform, followed by the addition of 2 mL of concentrated sulfuric acid. The formation of a reddish-brown ring indicated the presence of steroids. Tannins were detected by adding 5% ferric chloride solution dropwise to 2-3 mL of the test sample. The formation of a dark green precipitate indicated the presence of tannins.

Antibacterial Activity

Three to five colonies of *S. epidermidis* were isolated from slant cultures on Tryptic Soy Agar (TSA; Oxoid) and inoculated into Mueller-Hinton Broth (MHB; Oxoid), followed by incubation at 37 °C for 18 hours. The resulting bacterial suspension was adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Stock solutions of the extracts (100 mg/mL in 10% DMSO) were diluted with DMSO to obtain test concentrations of 250, 125, 62.5, and 31.25 mg/mL. Clindamycin phosphate (250 mg/mL) was used as the positive control. Antibacterial activity was assessed using the agar diffusion method with a well-perforation technique. A standardized bacterial suspension (20 µL) was inoculated into sterile Petri dishes containing 20 mL of Mueller-Hinton Agar (MHA; Oxoid), which was then allowed to solidify. Once solidified, wells with a 6 mm diameter were made using a sterile perforator, and each well was filled with 100 µL of the test solution. Before incubation, all test plates were kept at room temperature for one hour to allow diffusion of the test substances, then incubated at 37 °C for 24 hours. The diameter of the resulting inhibition zones was measured using a caliper and analyzed using ANOVA to assess differences in antibacterial effectiveness.

Synergistic Antibacterial Assay

The synergistic antibacterial interactions among *M. paniculata* leaf extract, *S. sonchifolius* leaf extract, *A. trigona* honey, and their combinations were evaluated using the agar diffusion method. The test media were prepared following the same procedure as in the antibacterial activity assay. A volume of 50 µL of each extract (500 mg/mL) was added into the same well for each combination, which included: *M. paniculata* + *S. sonchifolius* (KY), *S. sonchifolius* + *A. trigona* honey (YA), and *M. paniculata* + *A. trigona* honey (KA) (Table 1). For the combination of all three components (KYA), each extract was used at a concentration of 75 mg/mL (0.075 g extract in 1 mL of DMSO), with 33.3 µL of each solution placed into the same well. The

concentration of 75 mg/mL was selected based on preliminary antibacterial tests, which indicated that some extracts, particularly *S. sonchifolius*, already showed significant inhibitory effects at 31.25 mg/mL. Therefore, 75 mg/mL was chosen as a mid-range (sub-inhibitory) concentration that still demonstrated antibacterial activity but was not excessively high, allowing clearer evaluation of interaction effects (either synergistic or antagonistic). This concentration was also chosen to prevent dominance by any single extract in the combination, thereby ensuring that the observed interactions would better reflect the combined effects of the two or three tested substances. All test plates were incubated at 37°C for 24 hours, and the diameters of the inhibition zones were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC value was determined for the most potent individual test material or combination with antibacterial activity against *S. epidermidis* using the microdilution method, following the Clinical and Laboratory Standards Institute (CLSI 2012) guidelines. Each well of a microtiter plate was filled with 100 µL of the test material (at serial concentrations ranging from 250 to 7.8125 mg/mL) and 100 µL of the bacterial suspension, then incubated at 37°C for 24 hours. A negative control containing only media and a positive control containing media and bacterial suspension were included. All tests were performed in duplicate. The MIC was defined as the lowest concentration that showed no visible bacterial growth, as determined by optical density at 595 nm (OD₅₉₅). Subsequently, the MIC results were subcultured by inoculating 10 µL from each well onto agar plates to determine the lowest dose capable of inhibiting bacterial colony formation.

RESULT AND DISCUSSION

Antimicrobial resistance is now recognized as one of the major challenges in the treatment of infectious diseases. Therefore, the aim of this study was to analyze the antimicrobial interactions of various plant extracts against *S. epidermidis*, providing insights for practitioners and infectious disease specialists to establish strategic therapeutic foundations. The antibacterial interactions of *M. paniculata* extract, *S. sonchifolius* extract, *A. trigona* honey, and their combinations were evaluated to determine their interactive effects against *S. epidermidis*.

Extraction Yield and Phytochemical Content

Over the past decades, biologically and medically important phytochemicals found in leaf extracts and *A. trigona* honey have been shown to contain various compounds with notable biological and medicinal properties, including alkaloids, flavonoids, saponins, steroids, and tannins (Rachana *et al.* 2012). Phytochemicals play a crucial role in the defense mechanisms of plants against microbial infections (Yadav & Agarwala 2011). Alkaloids have been reported as one of the major components with significant antimicrobial activity (Abdelgadir & Van Staden 2013; Chandrasekaran 2008). Flavonoids are known to possess broad-spectrum antibacterial activity through multiple mechanisms, including inhibition of nucleic acid synthesis, disruption of cytoplasmic membrane function, interference with energy metabolism, reduction of bacterial adhesion for biofilm formation, and impairment of porins and membrane permeability (Panche *et al.* 2016; Wang *et al.* 2018; Kumar & Pandey 2016; Jucá *et al.* 2020; Donadio *et al.* 2021). Saponins, which are also present in many plants, are known to inhibit bacterial growth by disrupting the permeability of bacterial cell membranes (Khan *et al.* 2018; Winter 1994; Romo *et al.* 2016; Arabski *et al.* 2009). The integration of various phytochemicals in plant extracts may enhance their antibacterial potential. Alkaloids share similar antibacterial mechanisms with other phytochemical compounds, including inhibition of bacterial cell wall synthesis, metabolic pathways, nucleic acid and protein synthesis, as well as disruption of membrane permeability (Larghi *et al.* 2015). In this study, phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, steroids, and tannins in *M. paniculata* extract and *A. trigona* honey. However, steroids were absent in the *S. sonchifolius* extract, as shown in Table 2. These findings support the antibacterial potential of all three test materials against *S. epidermidis*.

Antibacterial Activity Results

M. paniculata leaves have demonstrated antibacterial activity against human infections due to their high phenolic and flavonoid content (Gautam *et al.* 2012). *S. sonchifolius* has also been reported to contain compounds with antibacterial activity. *S. sonchifolius* tubers are known to contain fructooligosaccharides and phenolic compounds (Lin *et al.* 2003; Ohyama *et al.* 1990), while the leaves contain a variety of kaurene-type diterpenoids, acetophenone-type phytoalexins, and melampolide-type sesquiterpene lactones (Hong *et al.* 2008). Similarly, honey from *Trigona* sp. has shown inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* at concentrations as low as 12.5% b/v. These studies provide a strong rationale for investigating the efficacy of combining these three antibacterial agents.

The antibacterial activity of the leaf extracts and *A. trigona* honey is presented in Table 3. All tested samples demonstrated strong inhibition against *S. epidermidis*. Among the plant extracts, *S. sonchifolius* extract exhibited the most potent antibacterial effect, while *A. trigona* honey showed the lowest average inhibition zone.

S. epidermidis exhibited varying sensitivity responses to the extracts and honey at the same concentrations. To evaluate the extent of the inhibitory potential of each sample, statistical analysis was conducted, as presented in Table 4. The effect of the extracts on the differences in inhibition zone diameters was analyzed using ANOVA. The ANOVA results showed a significance value of 0.000 ($p < 0.05$), indicating that there were statistically significant differences in the antibacterial potential among all samples at concentrations of 31.25, 125, and 250 mg/mL.

Synergistic Antibacterial Interaction Results

Distinct interaction patterns synergistic, additive, and antagonistic were observed between the honey and plant extracts, as shown in Table 5. The nature of

Table 2. Phytochemical screening results

Test Material	Phytochemical content				
	alkaloid	flavonoid	saponin	tannin	steroid
<i>M. paniculata</i>	+	+	+	+	+
<i>S. sonchifolius</i>	-	+	+	+	+
<i>A. trigona</i> honey	+	+	+	+	+

Table 3. Antibacterial activity

Test material	Inhibition zone diameter (mm) at concentration (mg/mL)			
	250	125	62.5	31.25
<i>M. paniculata</i>	20.00 ± 0.354	16.77 ± 0.39	10.85 ± 0.49	7.27 ± 0.46
<i>S. sonchifolius</i>	21.05 ± 0.636	18.25 ± 0.14	11.90 ± 0.56	8.25 ± 0.35
<i>A. trigona</i> honey	18.12 ± 0.884	13.07 ± 0.60	9.50 ± 0.14	6.37 ± 0.17
Clindamycin Phosphate (positive control)	22.37 ± 0.530			

Table 4. ANOVA results

Source of Variation	Sum of Squares	Df	Mean Square	F	Significance
Between groups	678.425	14	48.459	13.629	0.001
Within groups	53.333	15	3.556		
Total	731.757	29			

Table 5. Interaction effects of test material combinations on *Staphylococcus epidermidis*

Test Material Combination	Inhibition Zone Diameter (mm)
<i>S. sonchifolius</i> & <i>M. paniculata</i>	17.57±1.39
<i>S. sonchifolius</i> & <i>A. trigona</i> Honey	16.80 ±1.56
<i>M. paniculata</i> & <i>A. trigona</i> Honey	13.15±0.99
<i>S. sonchifolius</i> , <i>M. paniculata</i> & <i>A. trigona</i> Honey	20.05±0.35

the interaction was determined by comparing the inhibition zone diameters of individual agents (Table 4) with those of their combinations (Table 5). An interaction was considered additive when the combined effect equaled the sum of the individual effects, synergistic when the combined effect exceeded the sum, and antagonistic when the combined effect was less than the sum of the individual effects.

Based on the antibacterial activity results, both *S. sonchifolius* and *M. paniculata* extracts individually showed strong inhibitory effects against *S. epidermidis*, with maximum inhibition zones of 21.05±0.636 mm and 20.00±0.354 mm, respectively, at 250 mg/mL. The combination of these two extracts produced an inhibition zone of 17.57±1.39 mm, indicating an additive interaction, as the inhibitory effect was close to the average of the individual effects, without any biologically significant increase or decrease. In contrast, the combination of yacon extract and *A. trigona* honey showed an antagonistic interaction, with an inhibition zone of 16.80±1.56 mm, which was lower than that of either agent alone. This suggests potential interference from the honey's active compounds with those of the *S. sonchifolius* extract, possibly through cross-binding, local pH alteration, or competition in penetrating the bacterial cell membrane. Similarly, the interaction between kemuning extract and honey also exhibited an antagonistic effect, with an inhibition zone of 13.15±0.99 mm.

Interestingly, the combination of all three agents (*M. paniculata*, *S. sonchifolius*, and *A. trigona* honey) resulted in an additive effect (inhibition zone of 20.05±0.35 mm), which may be attributed to a balancing of active compounds that target different sites or act through complementary mechanisms. These findings suggest that *A. trigona* honey may interact negatively with the active compounds in *S. sonchifolius* and *M. paniculata* in dual combinations, but the interaction becomes neutral or additive when all three agents are combined. *S. sonchifolius* extract exhibited an additive interaction with *M. paniculata* extract, but an antagonistic one with *A. trigona*

honey. Similarly, an antagonistic effect was also observed between *M. paniculata* extract and honey. However, the overall combination of all three samples produced an additive interaction.

In this study, the leaf extract of *S. sonchifolius* exhibited the most potent antimicrobial activity among all tested samples, while *A. trigona* Honey showed the lowest average inhibition. For nearly a century, the concept of synergistic interaction between drugs and substances has been a central topic in biomedicine. Synergy is defined when the combined effect exceeds the sum of individual effects (Côté *et al.* 2016). Understanding drug interactions has become increasingly important, as complex diseases are often treated with multiple therapeutic combinations.

The concept of $1 + 1 = 2$ is not particularly compelling, and alternative outcomes such as $1 + 0 = 2$, $0 + 0 = 1$, or even $1 + 1 = 0$, though paradoxical, are widely discussed in academic discourse and civilizations worldwide. These models provide a simplified yet insightful framework for interpreting drug interactions, particularly synergy. Synergy is typically described as the combined effect of two or more agents that is greater than the expected additive effect of the individual agents. Returning to the example of $1 + 0 = 2$ (Berthoud 2013; Geary 2013), one may infer the presence of a synergistic interaction. Unfortunately, such interactions are not easily quantified in practice. Nevertheless, synergistic combinations hold the potential to maximize therapeutic outcomes while minimizing adverse effects or toxicity when applying specific pharmacological regimens (Greco *et al.* 1996; Fouquier & Guedj 2015). If two agents act synergistically, lower doses of each compound may be sufficient to achieve the desired effect, thereby reducing side effects. Our study demonstrated significantly different interactions (synergistic, additive, and antagonistic) between honey and crude plant extracts. Antagonism, the opposite of synergy, occurs when the combined effect of two or more substances is less than expected. Statistical analysis using ANOVA

Table 6. MIC and MBC results of *S. sonchifolius* leaf extract against *S. epidermidis*

Extract Concentration (mg/mL)	Bacterial Growth	
	MIC	MBC
7.8125	+	+
15.625	+	+
31.25	-	-
62.5	-	-
125	-	-
250	-	-
Clindamycin Phosphate (Positive Control)	-	-
Negative Control	-	-

Note: (+) indicates bacterial growth; (–) indicates no bacterial growth.

revealed that the differences in inhibition zone diameters among the tested combinations were significant ($p < 0.05$), confirming that each combination produced a distinct antibacterial response. Thus, the interaction between substances can influence antibacterial efficacy-either enhancing or diminishing the overall inhibitory activity.

MIC and MBC Values

The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of *S. sonchifolius* leaf extract, identified as the most potent antibacterial agent against *S. epidermidis*, were determined using test concentrations ranging from 7.8125 to 250 mg/mL, as presented in Table 6. Both the MIC and MBC values of the yacon leaf extract were found within the same concentration range, specifically $15.625 < x \leq 31.25$ mg/mL.

CONCLUSION

The interaction results among the test materials demonstrated significant differences. The combination of all three agents exhibited an antagonistic effect, while the combination of the two plant extracts showed a synergistic antibacterial interaction. However, the inhibitory activity produced by these combinations was not greater than that of the *S. sonchifolius* extract alone. Therefore, it can be concluded that *S. sonchifolius* leaf extract possesses strong potential as a standalone antibacterial agent against *S. epidermidis*.

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