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Antimicrobial Efficacy of *Pometia pinnata* Leaf Extracts Obtained by Ultrasound-Assisted Extraction with Choline Chloride:Citric Acid

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ABSTRACT

Bacterial and fungal infections, along with the rise of antimicrobial resistance, necessitate the exploration of novel natural antimicrobial agents. This study aimed to evaluate the efficacy of Pometia pinnata leaf extracts obtained using Ultrasound-Assisted Extraction (UAE) combined with a Natural Deep Eutectic Solvent (NADES) system based on choline chloride:citric acid, and to compare it with conventional methods. Various NADES molar ratios were tested to determine optimal conditions based on Total Phenolic Content (TPC) using the Folin–Ciocalteu method. Antimicrobial activity was assessed against Bacillus cereus, Shigella dysenteriae, and Malassezia furfur using disk diffusion and solid dilution methods. The results showed that the 1:3 molar ratio yielded the highest TPC (72.77±0.52 mg GAE/g). The NADES extract exhibited specific antibacterial activity against *B. cereus* (10.90±3.26 mm), while ethanol extracts were more effective against S. dysenteriae (11.78±0.98 mm). Against *M. furfur*, the NADES extract at 1,000 µg/disc demonstrated comparable activity to maceration at 6,000 µg/disc, with a Minimum Inhibitory Concentration (MIC) of 3,571 µg/mL. In conclusion, choline chloride:citric acid (1:3) NADES is an optimal green solvent for producing phenolic-rich extracts with specific and dose-efficient antimicrobial activity

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INTRODUCTION

Infectious diseases caused by pathogenic bacteria and fungi remain a leading cause of morbidity and mortality worldwide, imposing a substantial burden on global healthcare systems (World Health Organization, 2022). The emergence and spread of antimicrobial resistance (AMR) have further complicated the management of these infections, rendering many conventional antibiotics ineffective (Review on Antimicrobial Resistance, 2016). Among the diverse range of pathogens, *Bacillus cereus* (a Gram-positive foodborne agent), *Shigella dysenteriae* (a Gram-negative enteric pathogen), and *Malassezia furfur* (a lipophilic skin fungus) represent distinct clinical challenges spanning food safety, gastrointestinal health, and dermatology (Diongue et al., 2018; Habibou et al., 2024; Yennie et al., 2022). The selection of these specific pathogens in this study is driven by the need for broad-spectrum natural alternatives that can address infections across different body systems, a "common thread" aligning with the search for versatile antimicrobial agents.

In response to AMR, plants are increasingly explored as reservoirs of bioactive secondary metabolites. *Pometia pinnata*, commonly known as Matoa, is an endemic Indonesian plant traditionally used to treat various ailments, including diarrhea, dysentery, and skin conditions (Sidoretno & Gustari, 2021). This ethnopharmacological background provides a strong rationale for investigating its efficacy against enteric pathogens like *S. dysenteriae* and skin pathogens like *M. furfur*. The therapeutic potential of Matoa leaves is attributed to their secondary metabolite content, particularly phenolic compounds and flavonoids such as quercetin and kaempferol, which are known to possess antimicrobial mechanisms (Putri et al., 2023; Utari et al., 2019). However, while previous studies have confirmed activity against *Staphylococcus aureus* and *Trichophyton mentagrophytes*, the efficacy of *P. pinnata* extracts against *B. cereus* and *M. furfur*. specifically when extracted using green solvents, remains largely underexplored (Litaay, 2023; Sidoretno & Gustari, 2021).

The efficacy of plant-derived antimicrobials is intrinsically linked to the extraction methods employed (Bitwell et al., 2023). Conventional methods like maceration, percolation and reflux often require toxic organic solvents and prolonged heating, which risks degrading thermolabile compounds (Abubakar & Haque, 2020; Kumar et al., 2021). To address these limitations, green extraction technologies such as Ultrasound-Assisted Extraction (UAE) combined with Natural Deep Eutectic Solvents (NADES) have gained prominence. UAE utilizes acoustic cavitation to disrupt plant cell walls, facilitating the release of intracellular compounds while

significantly reducing extraction time and solvent consumption (Chemat et al., 2017). NADES are biodegradable, non-toxic, and possess high solvation capacity for polar compounds (Cannavacciuolo et al., 2023; Dai et al., 2013). Despite the promise of NADES, critical knowledge gaps remain. It is currently unclear whether specific NADES systems, such as choline chloride:citric acid, perform better against Gram-positive versus Gram-negative bacteria due to differences in cell wall structure, or whether they increase potency per dose compared to conventional solvents. Furthermore, the potential trade-offs involving viscosity and pH in NADES extracts and their impact on antimicrobial readouts need further elucidation.

Choline chloride:citric acid-based NADES, in particular, has shown exceptional ability to extract phenolic acids and flavonoids due to its high polarity and hydrogen bonding capacity (Cannavacciuolo et al., 2023; Kartikaningsih et al., 2024). Therefore, this study aims to evaluate the antimicrobial activity of *Pometia pinnata* leaf extracts obtained through UAE using choline chloride:citric acid against *Bacillus cereus*, *Shigella dysenteriae*, and *Malassezia furfur*, comparing its efficacy with conventional methods. This study provides new insight into effectiveness of NADES-based green extraction systems across different classes of microorganisms, as well as their potential for dose-efficient antimicrobial applications.

METHODS

Research Design

This study employed an experimental laboratory design to evaluate the antimicrobial activity of *Pometia pinnata* leaf extracts obtained using different extraction methods and solvent systems.

Materials and Instruments

The plant identity was authenticated at the Laboratory of FMIPA, Universitas Andalas, Padang Identification No: 865/K-ID/ANDA/XII/2023. The materials included Matoa leaf powder, Folin-Ciocalteu reagent (Merck), gallic acid (Merck), sodium hydroxide (NaOH) (Merck), choline chloride (Chengdu Chemical), citric acid (Tianjin Chemical), ethanol (Merck), aquabidest (Ikapharmindo), dimethyl sulfoxide (DMSO) (Merck), Mueller-Hinton Agar (MHA) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), Tetracycline disk (30 μg), ketoconazole disk (30 μg). The test microorganisms used in this study were *Bacillus cereus* ATCC 14579, *Shigella dysenteriae* YP13233, and *Malassezia furfur* YPC12214. Instruments included an ultrasonic bath (Branson 1800), rotary evaporator (Heidolph), autoclave (Nesco) laminar air flow, analytical balance (Kenko), UV-Vis spectrophotometer (Shimadzu) and standard glassware (Pyrex).

Standardization

The leaf powder was standardized to ensure compliance with the Indonesian Herbal Pharmacopoeia Kementerian Kesehatan Republik Indonesia, 2017). The evaluation included specific parameters, namely organoleptic properties (color, odor, and taste) and the determination of water-soluble and ethanol-soluble extractive values. Non-specific parameters comprised the assessment of drying loss, moisture content, total ash content, and acid-insoluble ash content (Sutomo et al., 2021).

Preparation of NADES

Natural Deep Eutectic Solvents (NADES) were prepared by mixing choline chloride (hydrogen bond acceptor) and citric acid (hydrogen bond donor) at molar ratios of 1:1, 1:2, 2:1, 1:3, and 3:1. The mixtures were heated and stirred at 80°C and 500 rpm for 60 minutes until a clear and homogeneous liquid was obtained. Subsequently, distilled water (30% w/w) was added to reduce viscosity and improve handling properties, followed by further stirring until a uniform solution was achieved (Cannavacciuolo et al., 2023).

Extraction Procedures

Two extraction methods were employed to obtain Matoa leaf extracts, namely UAE and conventional maceration.

1. **Ultrasound-Assisted Extraction (UAE)**

UAE was performed by mixing 2 g of leaf powder with 40 mL of solvent (96% ethanol or choline chloride:citric acid) in an ultrasonic bath operating at a frequency of 40 kHz. The extraction was conducted at room temperature for 25 minutes. The resulting mixture was centrifuged at 4.000 rpm for 10 minutes, and the supernatant was collected as the extract (Hasan et al., 2025).

2. **Maceration**

Maceration was carried out by soaking 5 g of leaf powder in 200 mL of 96% ethanol (1:40 w/v) at room temperature for 24 hours with occasional stirring to facilitate solvent penetration and compound diffusion. The mixture was then filtered using Whatman filter paper, and the filtrate was concentrated using a rotary evaporator followed by a water bath to obtain the crude extract (Abubakar & Haque, 2020).

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method with slight modifications. A calibration curve was prepared using gallic acid standard solutions at concentrations ranging from 15 to 100 µg/mL. The maximum absorbance wavelength was determined at 738 nm by scanning within the range of 400–800 nm For the analysis, 1 mL of the extract solution was mixed with 5 mL of 7.5% (v/v) Folin–Ciocalteu reagent and allowed to stand for 8 minutes at room temperature. Subsequently, 4 mL of 1% (w/v) NaOH solution was added, and the mixture was incubated in the dark for 60 minutes at room temperature. The absorbance was then measured using a UV–Vis spectrophotometer. The total phenolic content was calculated based on the gallic acid calibration curve and expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g). Kementerian Kesehatan Republik Indonesia, 2017). The molar ratio of choline chloride:citric acid that yielded the highest TPC was subsequently selected for antimicrobial activity testing.

Antimicrobial Activity Testing

The antimicrobial activity of the extracts was evaluated using the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, with slight modifications (Balouiri et al., 2016). Mueller-Hinton Agar (MHA) plates were used for antibacterial assays, while Sabouraud Dextrose Agar (SDA) plates were employed for antifungal assays. Each Petri dish was prepared with 15 mL of agar medium to ensure consistent agar thickness.

The agar surfaces were uniformly inoculated with microbial suspensions standardized to 0,5 McFarland (approximately 1,5 × 10^8 CFU/mL). Sterile paper disks (6 mm in diameter) were impregnated with 20 µL of each test solution and aseptically placed onto the inoculated agar surface. Tetracycline (30 µg/disk) and ketoconazole (30 µg/disk) were used as positive controls for antibacterial and antifungal assays, respectively. Dimethyl sulfoxide (DMSO) served as the negative control for conventional extracts, while a blank NADES solution (choline chloride:citric acid, 1:3) without plant material was used as the negative control for NADES extracts to account for any intrinsic antimicrobial effect of the solvent.

The plates were incubated at 37°C for 18–24 hours for bacteria and at 32°C for 3–5 days for fungi. Following incubation, the inhibition zones were measured as the diameter of the clear zone, including the disk diameter, and expressed in millimeters (mm). For NADES samples, the net inhibition zone was calculated by subtracting the inhibition zone of the blank NADES control from that of the sample to ensure that the observed activity was solely attributable to the extracted bioactive compounds. All experiments were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the agar dilution method in accordance with CLSI guidelines, with slight modifications (Weinstein & Lewis, 2020; Wiegand et al., 2008). Serial two-fold dilutions of the NADES extract were prepared and incorporated into molten Mueller–Hinton Agar (MHA) for bacterial assays and Sabouraud Dextrose Agar (SDA) for fungal assays. The agar-extract mixtures were then poured into sterile Petri dishes and allowed to solidify. The solidified media were spot-inoculated with 10 µL of standardized microbial suspension (adjusted to 0.5 McFarland standard for both bacteria and fungi). Agar plates without extract were used as growth controls. The inoculated plates were incubated at 37°C for 18–24 hours for bacteria and at 32°C for 3–5 days for fungi. Following incubation, microbial growth was evaluated visually. The MIC was determined as the lowest concentration of the extract that completely inhibited visible microbial growth, representing the transition between concentrations that permitted growth and those that showed no growth, in comparison with the growth control (Balouiri et al., 2016).

Data analysis

All experimental data were expressed as mean ± standard deviation (SD) from triplicate measurements. Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate significant differences among groups, followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Statistical analysis was performed using SPSS version 29.

RESULTS OF STUDY

Simplisia Standardization Results

The standardization of *Pometia pinnata* leaf powder was conducted to ensure its quality in accordance with the established pharmacopeial requirements. The results of both specific and non-specific parameters are presented in Table 1. For the specific parameters, organoleptic evaluation showed that the powder was slightly fine in texture, greenish in color, possessed a characteristic odor and had a bitter taste. The extractive values indicated that the water-soluble extractive was 17.561%, while the ethanol-soluble extractive was 25.938%. Both values complied with the minimum requirements (≥16% for water-soluble and ≥8% for ethanol-soluble extractives). The higher ethanol-soluble extractive value suggests that the leaf powder predominantly contains semi-polar to non-polar compounds compared to polar constituents.

Regarding non-specific parameters, the drying loss and moisture content were 8.2% and 7.2%, respectively, both within the acceptable limit of ≤10%, indicating adequate drying and low susceptibility to microbial growth. The total ash content was 8.9%, which met the required limit (≤16, 6%), reflecting an acceptable level of total inorganic content. However, the acid-insoluble ash content was 2.2%, slightly exceeding the standard limit (≤2%), which may indicate the presence of residual inorganic contaminants such as soil or sand introduced during sample handling or processing (Sutomo et al., 2021).

Table 1. Results of Matoa Leaf Powder Standardization

Parameter	Results (%)
Water-soluble extract content	17.561
Ethanol-soluble extract content	25.938
Drying loss	8.2
Moisture content	7.2
Total ash content	8.9
Acid-insoluble ash content	2.2

Total Phenolic Content Analysis

Table 2. Total Phenolic Content of Pometia pinnata Leaf Extracts

Extract Type	Molar Ratio	Total Phenolic Content (mg GAE/g powder)
Conventional Extracts		
Maceration Ethanol	-	17.445 ± 0.2797
UAE Ethanol	-	66.252 ± 0.0870
NADES-UAE Extracts		
Choline Chloride:Citric Acid	1:3	72.774 ± 0.5217
Choline Chloride:Citric Acid	1:1	69.933 ± 0.3921
Choline Chloride:Citric Acid	2:1	59.238 ± 0.2795
Choline Chloride:Citric Acid	3:1	51.180 ± 0.8265
Choline Chloride:Citric Acid	1:2	41.354 ± 0.3292

The total phenolic content (TPC) of Matoa leaf extracts was determined using the Folin–Ciocalteu method. The calibration curve of gallic acid showed good linearity with the regression equation y= 0.0115x+0.1101 and R^2 of 0.9961. The TPC values of the extracts are presented in Table 2. The results showed significant variation depending on the extraction method and solvent used. The highest TPC was observed in the NADES-UAE extract with a choline chloride:citric acid molar ratio of 1:3 (72.774 ± 0.5217 mg GAE/g), while the lowest value was

found in the conventional maceration extract (17.445 ± 0.2797 mg GAE/g). Statistical analysis using one-way ANOVA revealed a significant difference among all extract groups ($p < 0.05$), indicating that both extraction method and solvent system significantly influenced the phenolic content.

Antibacterial Activity Results

The antibacterial activity of *Pometia pinnata* leaf extracts was evaluated against *Bacillus cereus* (Gram-positive) and *Shigella dysenteriae* (Gram-negative) using the disk diffusion method. The results are presented in Table 3. Against *B. cereus*, conventional ethanol extracts obtained by maceration and UAE showed no detectable inhibition. In contrast, the NADES-UAE extract (choline chloride:citric acid, 1:3) exhibited antibacterial activity with an inhibition zone of 10.90 ± 3.26 mm. For *S. dysenteriae*, all extracts demonstrated antibacterial activity. The conventional maceration extract showed the highest inhibition zone (11.78± 0.98 mm), followed by the UAE ethanol extract (10.83 ± 2.68 mm). The NADES-UAE extract exhibited moderate activity with an inhibition zone of 9.66 ± 0.33 mm. These results indicate that the antimicrobial effectiveness of the extracts varies depending on the microorganism, with NADES-UAE showing selective against *Bacillus cereus*, while ethanol extracts demonstrated better activity against *Shigella dysenteriae*. Overall, the antibacterial activity varied depending on the extraction method and solvent system used (Widyanti & Maryati, 2023).

Table 3. Inhibition Zones of Pometia pinnata Extracts Against Bacteria

Extract Type	Concentration (ug/disk)	Inhibition Zone *B. cereus* (mm)	Inhibition Zone *S. dysentery* (mm)
Conventional Maceration (Ethanol)	30,000	-	11.78 ± 0.98
Conventional UAE (Ethanol)	1,000	-	10.83 ± 2.68
Choline Chloride:Citric Acid	1,000	10.90 ± 3.26 (Net)*	9.66 ± 0.33 (Net)*
Positive Control (Tetracycline)	30	31.13 ± 1.91	29.97 ± 1.46
Negative Control (DMSO)	-	-	-
Control NADES	-	20.23 ± 0.58	21.93 ± 1.27

Note:
*The inhibition zone for NADES extract is presented as the "Net" value, calculated by subtracting the inhibition zone of the Blank NADES Control from the total inhibition zone of the sample (Total Sample Zone - Blank Control Zone = Net Zone).

Antifungal Activity Results

The antifungal activity of *Pometia pinnata* leaf extracts was evaluated against *Malassezia furfur* using the disk diffusion method. The results are presented in Table 4. The conventional maceration extract at a concentration of 6,000 µg/disc produced an inhibition zone of 9.78 ± 0.36 mm. The NADES-UAE extract (choline chloride:citric acid, 1:3) at a lower concentration of 1,000 µg/disc showed a comparable inhibition zone of 9.37 ± 0.71 mm. In contrast, the conventional UAE ethanol extract exhibited the lowest antifungal activity, with an inhibition zone of 7.48 ± 1.05 mm. Notably, the NADES extract exhibited comparable antifungal activity at a lower concentration, suggesting improved dose-efficiency compared to conventional extraction methods.

Table 4. Inhibition Zones of Pometia pinnata Extracts Against Malassezia furfur

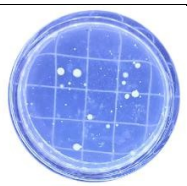
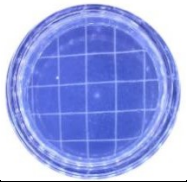
Extract Type	Concentration (ug/disk)	Inhibition Zone (mm)
Conventional Maceration (Ethanol)	6,000	9.78 ± 0.355
Conventional UAE (Ethanol)	1,000	7.48 ± 1.054
Choline Chloride:Citric Acid	1,000	9.37 ± 0.709
Positive Control (Ketoconazole)	30	26.88 ± 3.019 (Net)*
Negative Control (DMSO)	-	-
Control NADES	-	25.48 ± 0.369

Note:
* The inhibition zone for NADES extract is presented as the "Net" value, calculated by subtracting the inhibition zone of the Blank NADES Control from the total inhibition zone of the sample (Total Sample Zone - Blank Control Zone = Net Zone).

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the agar dilution method. The results are presented in Table 5. For the antibacterial evaluation, choline chloride: citric acid extract with ratio 1:3 did not show inhibitory activity against *Bacillus cereus* and *Shigella dysenteriae*, as visible microbial growth was observed at all tested concentrations, including the highest concentration (500 mg/mL). In contrast, antifungal activity against *Malassezia furfur* was observed. The MIC value was determined as 3,571 µg/mL, representing the lowest concentration at which no visible fungal growth was detected on the agar surface.

Table 5. Determination of Minimum Inhibitory Concentration (MIC) of NADES UAE Extract Against *Malassezia furfur*

Dilution of Extract (x)	Concentration of Extract (µg/ml)	Visual Observation	
15 x	3,333	Growth	
14 x	3,571	No growth	

DISCUSSION

This study demonstrated that both extraction method and solvent system significantly influenced the total phenolic content (TPC) and antimicrobial activity of *Pometia pinnata* leaf extracts. Among all tested conditions, the NADES-UAE extract with a choline chloride:citric acid molar ratio of 1:3 exhibited the highest TPC, indicating its efficiency in extracting phenolic compounds. This finding is consistent with previous studies reporting that NADES based on organic acids possess higher polarity, thereby enhancing the solubility of phenolic compounds (Cannavacciuolo et al., 2023; Dai et al., 2013). In addition, the acidic nature of the solvent system may promote disruption of plant cell walls and vacuoles, facilitating the release of intracellular phenolics (Kartikaningsih et al., 2024).

A notable finding in this study was the significant antimicrobial activity exhibited by the blank NADES control, which produced clear inhibition zones against all tested microorganisms. This phenomenon indicates that the NADES solvent matrix itself possesses intrinsic antimicrobial properties. The mechanism underlying this activity is primarily attributed to the acidic nature of citric acid as the hydrogen bond donor. The low pH environment created by the solvent can disrupt the proton motive force across the microbial cell membrane, leading to energy depletion and cell death (Dai et al., 2013). Furthermore, organic acids like citric acid can act as chelating agents, sequestering metal ions essential for microbial enzyme function and membrane stability (Radošević et al., 2016). The strong hydrogen-bonding network within the NADES may also interfere with the cell wall integrity of pathogens, enhancing membrane permeability (Cannavacciuolo et al., 2023). Consequently, while the "net" inhibition zone was calculated to isolate the specific contribution of the phenolic compounds, the presence of the NADES solvent suggests a dual mechanism of action: it serves as an effective extraction medium while simultaneously contributing to the antimicrobial effect.

The elevated TPC observed in NADES extracts was associated with enhanced antimicrobial activity, particularly against *Bacillus cereus* and *Malassezia furfur*. The NADES extract demonstrated antibacterial activity against *B. cereus*, whereas conventional ethanol extracts showed no inhibition. This result may be attributed to the ability of phenolic compounds to disrupt bacterial cell membranes, alter permeability, and interfere with enzymatic systems (Nazzaro et al., 2013). In contrast, all extracts exhibited antibacterial activity against *Shigella dysenteriae*, with conventional ethanol extracts showing slightly higher inhibition zones. This difference may be explained by the structural characteristics of Gram-negative bacteria, which possess an outer membrane containing lipopolysaccharides that can limit the penetration of highly polar compounds (Widyanti & Maryati, 2023). These findings offer critical insights for solvent design: choline chloride: citric acid is preferable when targeting polar phenolic metabolites intended for action against Gram-positive pathogens, whereas less polar solvent systems may be required to combat Gram-negative infections effectively (Cannavacciuolo et al., 2023; Dai et al., 2013).

Regarding antifungal activity, the NADES-UAE extract exhibited comparable inhibition against *Malassezia furfur* at a lower concentration compared to the conventional maceration extract, suggesting a higher efficiency in extracting antifungal bioactive compounds. This dose-efficiency against *M. furfur* suggests that the NADES system significantly enhances the bioavailability or stability of antifungal compounds, such as flavonoids and phenolic acids. The acidic nature of the choline chloride:citric acid solvent may also inhibit hyphal growth and spore germination, providing a dual mechanism of action alongside the extracted phenolics (Dai et al., 2013).

The MIC results further supported these findings. The NADES extract showed inhibitory activity against *M. furfur*, with complete inhibition observed at 3,571 µg/mL. However, no MIC value was obtained for antibacterial activity within the tested concentration range, indicating that higher concentrations may be required to achieve complete inhibition in solid media. This discrepancy between diffusion-based assays and dilution methods has been previously reported and may be influenced by differences in compound diffusion, solubility, and interaction with the agar matrix (Balouiri et al., 2016).

CONCLUSIONS AND RECOMMENDATION

This study demonstrates that the antimicrobial activity of *Pometia pinnata* leaf extracts is strongly influenced by the extraction method and solvent system. The choline chloride: citric acid (1:3) NADES combined with UAE was the most effective approach for enhancing total phenolic content, indicating extraction efficiency for polar compounds. The NADES extract exhibited selective activity against the Gram-positive *Bacillus cereus*, while ethanol extracts were more effective against the Gram-negative *Shigella dysenteriae*, highlighting the role of solvent polarity in determining antimicrobial spectrum. Notably, the NADES extract showed dose-efficient antifungal activity against *Malassezia furfur*, achieving comparable inhibition at lower concentrations. However, the absence of antibacterial MIC suggests limited bactericidal potency under the tested conditions. Overall, NADES-UAE represents a promising green extraction strategy for selective and efficient antimicrobial applications.

Further studies should focus on the identification of active compounds, mechanistic evaluation, standardization of extract concentrations, and in vivo safety assessment, along with formulation development to optimize its antifungal potential.

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Conflicts of interest

The authors declare no conflict of interest

Ethics approval and consent to participate

Not applicable

Consent for publication
Not applicable.

Availability of data and materials
The datasets analyzed during the current study are available from the corresponding author on reasonable request

Artificial Intelligence-Assisted Technology
Not applicable.

Authors' contributions:
Fasri Dian Safitri performed the antifungal experiments, collected and analyzed the data, and drafted the original manuscript. **Venty Wahyu Tariyani** performed the antibacterial experiments, collected and analyzed the data and assisted in the manuscript preparation. **Suhaera supervised** the research methodology for the antifungal studies and assisted in data analysis and manuscript revisi. **Nahrul Hasan** conceptualized the study, supervised the research methodology and analysis, acquired the funding, and edited the final manuscript. **Putri Khaerani Cahyaningrum** assisted in the sample preparation, data collection and literature review

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