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# Preliminary In Vitro Screening of a 70% Ethanol Extract of Matoa (*Pometia pinnata*) Fruit Peel against *Staphylococcus epidermidis* and *Trichophyton mentagrophytes* Using the Disc Diffusion Method

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***ABSTRACT***. *Matoa (Pometia pinnata) fruit peel is an agricultural by-product whose activity against skin-associated microorganisms remains insufficiently characterized. This study evaluated a 70% ethanol extract of matoa fruit peel against Staphylococcus epidermidis ATCC 12228 and Trichophyton mentagrophytes ATCC 18748 using the disc diffusion method at 15%, 20%, 25% and 30% (w/v). Chloramphenicol and ketoconazole served as positive controls, while 10% dimethyl sulfoxide served as the negative control. Three technical replicates were performed per treatment using a single extraction batch. Mean widths of the clear zones beyond the discs ranged from 6.33 to 9.33 mm for S. epidermidis and from 14.00 to 18.00 mm for T. mentagrophytes. No clear zone beyond the disc was observed with the negative control. Analysis of variance showed no significant differences among extract concentrations for either organism ($p > 0.05$). These preliminary findings require cautious interpretation because the replicates were technical rather than biological. Further studies using independent extraction batches and minimum inhibitory concentration testing are needed.*

***Keywords:*** *Pometia pinnata, disc diffusion, antimicrobial screening, Staphylococcus epidermidis, Trichophyton mentagrophytes.*

## INTRODUCTION

Bacterial and fungal infections of the skin remain a substantial clinical burden, and they fall disproportionately on tropical, resource-limited settings. In a four-year cross-sectional study of dermatology presentations in Palau, superficial fungal infection was the second most prevalent diagnosis, affecting 24.5% of patients [1]. In parallel, antimicrobial resistance continues to erode the utility of existing agents: an estimated 4.71 million deaths were associated with bacterial antimicrobial resistance worldwide in 2021, and this figure is forecast to rise [2]. Together these trends sustain interest in new antimicrobial leads, including those recoverable from agricultural by-products.

*Staphylococcus epidermidis* is a member of the normal skin microbiota that can behave as an opportunistic pathogen, particularly on indwelling devices, where biofilm formation is its principal virulence determinant [3]. It is important to be precise about strain selection here. The strain used in the present study, *S. epidermidis* ATCC 12228, is a sequenced, non-biofilm-forming, non-infection-associated isolate in which the *ica* operon responsible for polysaccharide intercellular adhesin synthesis is absent [4]. Biofilm phenotypes therefore cannot be addressed with this strain, and no anti-biofilm claim is made in this work.

*Trichophyton mentagrophytes* is a dermatophyte that infects keratinised tissue. Its clinical management is complicated by rising terbinafine resistance and by the absence of published minimum inhibitory concentration breakpoints for dermatophytes, which leaves culture-based susceptibility results difficult to interpret [5].

Matoa (*Pometia pinnata* J.R.Forst. & G.Forst., Sapindaceae) is widely distributed across Indonesia [6]. Qualitative phytochemical screening of dried matoa fruit peel has detected flavonoids, tannins and saponins, while alkaloids, quinones, steroids and triterpenoids were not detected [7]. This specific profile, rather than a generic list of plant secondary metabolites, is the rationale for examining the peel.

The existing evidence base for *P. pinnata* is unevenly distributed between plant parts, and the following summary makes the gap explicit:

(1) Leaves have been studied most. A 70% ethanol leaf extract inhibited *Staphylococcus aureus* and *Escherichia coli*, with zones of 18.22 mm and 10.87 mm respectively at only 10% [8]. An ethanol leaf extract inhibited *T. mentagrophytes* with zones of 23.05–25.05 mm at 10–30% [9].

(2) Fruit peel has been studied less, and the reported activity is weaker. A 96% ethanol peel extract gave zones of 6.6–8.3 mm against *S. aureus* by well diffusion at 20–60%, without a consistent rise across concentrations [10]. A peel extract tested

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against *Streptococcus mutans* produced zones of only 0.86–1.63 mm at 75–100% and no zone at 25–50% [11].

(3) Neither *S. epidermidis* nor *T. mentagrophytes* has been tested against the fruit peel, although both are skin-associated and the peel is a discarded by-product.

The contribution of this study is therefore narrow and specific: it is the first disc-diffusion screen of a matoa fruit peel extract against these two skin-associated organisms that we were able to identify, and it pairs a Gram-positive commensal with a dermatophyte under a single extraction and assay protocol. It is not a claim of superior activity, and no priority claim is made beyond the literature searched. The concentration range of 15–30% (w/v) was selected to sit above the 25–50% range at which peel extracts have given no measurable zone [11] while remaining below the solubility ceiling of a crude extract in 10% dimethyl sulfoxide (DMSO).

The objective was to measure, under one protocol, the inhibition-zone response of *S. epidermidis* ATCC 12228 and *T. mentagrophytes* ATCC 18748 to a 70% ethanol extract of matoa fruit peel at four concentrations, against vehicle and antimicrobial controls, and to report that response with uncertainty proportionate to a preliminary screen.

## RESEARCH METHODS

### Plant Material and Botanical Authentication

Matoa (*Pometia pinnata*) fruits were collected from a single tree in Takalar, South Sulawesi, Indonesia, on 30 January 2026. Approximately 2 kg of fruit, including the peel and flesh, was collected during a single collection event. The fruits were visually inspected, and visibly damaged fruits were discarded. The selected fruits exhibited varying peel colors, ranging from greenish-brown to reddish-purple and dark brown. These visual characteristics were used during fruit selection; however, a standardized maturity classification was not established.

The selected fruits were washed under running water. The peels were manually separated from the fruit flesh using a knife and washed again before processing.

### Preparation of Dried Peel and Extraction

The separated matoa peels were air-dried at room temperature for 24 h and subsequently oven-dried at 40 °C for 3 × 24 h. The dried peels were ground using a blender and passed through a 60-mesh sieve. A total of 250 g of powdered matoa peel was obtained and stored in a closed container at approximately 26 °C for two days before extraction.

Extraction was performed by maceration using 70% ethanol at a powder-to-solvent ratio of 1:10 (w/v). Maceration was conducted over three successive 24-h periods, using 2.5 L of fresh 70% ethanol for each cycle, giving a total solvent volume of 7.5 L. After each 24-h period, the mixture was filtered using Whatman filter paper, and the remaining plant material was re-macerated with fresh solvent. The filtrates from all three cycles were combined and concentrated using a rotary evaporator at 48 °C and a pressure of 200 mmHg. The concentrated extract was subsequently placed in an oven at 40 °C for 24 h to further reduce the residual solvent. The resulting thick extract was stored in a closed container in a refrigerator until use.

The extraction yield was 17%, expressed as the percentage of concentrated extract obtained relative to the initial mass of powdered matoa peel.

### Preparation of Test Solutions

A 30% (w/v) stock was prepared by combining 3 g of crude extract with 10% DMSO to a final volume of 10 mL, equivalent to 300 mg $mL^{-1}$. Working concentrations of 25%, 20% and 15% (250, 200 and 150 mg $mL^{-1}$) were prepared from this stock by transferring 8.33, 6.67 and 5.00 mL respectively and making up to 10 mL with 10% DMSO. These dilutions are arithmetically consistent with the stated stock concentration. With a 10 µL application volume, the nominal doses were 1.5, 2.0, 2.5 and 3.0 mg per disc for 15%, 20%, 25% and 30% respectively. The term nominal is used deliberately: the fraction of crude extract actually in solution was not determined analytically, and complete dissolution of a crude extract at 300 mg $mL^{-1}$ in 10% DMSO cannot be assumed.

### Test Microorganisms and Inoculum Preparation

*Staphylococcus epidermidis* ATCC 12228 and *Trichophyton mentagrophytes* ATCC 18748 were obtained from Universitas Brawijaya, Malang, East Java, Indonesia. The cultures used were one month old and had been stored at 4 °C.

For the antibacterial assay, a suspension of *S. epidermidis* was prepared in sterile physiological saline and adjusted visually to a turbidity equivalent to a 0.5 McFarland standard, corresponding nominally to approximately 1–2 × $10^8$ CFU $mL^{-1}$. The suspension was spread over the surface of Mueller–Hinton agar (MHA) with an approximate agar depth of 4 mm.

For the antifungal assay, *T. mentagrophytes* was subcultured on Sabouraud dextrose agar (SDA) and incubated at 25–28 °C for 4–7 days. The resulting colony material was suspended in sterile physiological saline and spread over the SDA surface. The fungal inoculum was not standardized by quantitative conidial counting; therefore, the conidial concentration and the relative proportions of

conidia and hyphal fragments were not determined. This limitation should be considered when interpreting the antifungal results.

**Disc Diffusion Assays**

Aliquots of 10 µL of each extract concentration were applied to sterile 6 mm paper discs and allowed to absorb for approximately 15 min before placement on the inoculated surface. Discs were placed approximately 24 mm apart. Antibacterial plates were incubated at 37 °C for 24 h; antifungal plates at 25–28 °C for 7 days.

The photographic record (Figures 1 and 2) shows that all four extract concentrations were placed together on one plate, while the positive and negative controls were placed together on a second plate. This layout, rather than a fully randomized allocation of single discs to plates, determines the experimental unit and the appropriate analysis, and is addressed below (Experimental Unit and Replication; Statistical Analysis).

**Controls**

A chloramphenicol capsule formulation labeled as containing 250 mg was used as the positive control in the antibacterial assay, with 30 µL applied per disc. For the antifungal assay, 100 mg of powdered ketoconazole tablet formulation, labeled as containing 200 mg of active ingredient per tablet, was mixed with 5 mL of dimethyl sulfoxide (DMSO). This preparation corresponded to a nominal tablet-powder concentration of 2% (w/v), and 30 µL was applied per disc. A 10% DMSO solution served as the negative control in both assays.

The amount of active chloramphenicol delivered per disc could not be determined from the available preparation records. Similarly, the actual dissolved concentration of ketoconazole could not be confirmed from the mass of tablet powder alone. Consequently, the active antimicrobial dose delivered per disc was not established for either positive control. No separate control was included to assess the potential contribution of residual ethanol from the extract preparation. Furthermore, the positive and negative controls were tested on separate agar plates from the extract treatments. These limitations mean that comparisons between the extract and the reference agents are descriptive and cannot establish their relative antimicrobial potency.

**Measurement of Inhibition Zones**

The inhibition zones were measured using a caliper along two perpendicular axes, and the two measurements were averaged to obtain the reported value. Measurements were taken from the outer edge of each 6-mm paper disc to the outer boundary of the clear zone. Accordingly, the reported values represent clear-zone measurements extending beyond the disc edges and do not include the diameter of the paper discs. The distance between adjacent discs was not measured or recorded; therefore, potential effects of disc spacing and overlapping inhibition zones could not be evaluated.

For the antifungal assay, the observations recorded on day 7 were used for the analysis reported in this study. Because the clear areas around adjacent discs were not always clearly separated, individual antifungal measurements should be interpreted cautiously. The extract treatments consisted of four concentrations (15%, 20%, 25%, and 30%), with the discs arranged on the agar surface. Positive and negative controls were tested on separate agar plates from the extract treatments. Representative photographs of the extract treatments and controls are provided in Figure 2.

The disc diffusion assays were conducted as preliminary *in vitro* screening and were not performed in accordance with the standardized susceptibility-testing procedures described in CLSI M02 for bacteria or CLSI M38 for filamentous fungi. Therefore, the measured clear-zone widths are reported as observations under the experimental conditions used and are not interpreted using clinical susceptibility breakpoints.

The original measurements were retained as recorded, and no blanket addition or subtraction of the 6-mm disc diameter was applied. The negative control was recorded as 0 to indicate that no inhibition was observed beyond the disc edge. Because the original measurement records did not permit independent verification of every recorded value, the measurements were not retrospectively converted to total inhibition-zone diameters or adjusted clear-zone widths. These limitations should be considered when interpreting the results presented in Tables 1 and 2.

**Experimental Unit and Replication**

Each extract concentration was tested in triplicate. All replicates were derived from a single extract batch prepared from fruit collected from one tree during a single collection event. Therefore, the replicates represent technical replicates rather than independent biological replicates of the plant material or extraction process.

The documented antibacterial assay layout consisted of four extract-treatment discs (15%, 20%, 25%, and 30% w/v) on one agar plate, while the positive and negative control discs were placed on a separate plate. This arrangement was repeated across three replicates, resulting in six agar plates for the antibacterial assay. Because all four extract concentrations were placed on the same plate within each replicate, the plate may be considered a block for the extract-treatment comparisons. Treating the

four discs on a plate as independent experimental units without accounting for this shared plate would risk pseudoreplication. In addition, the controls were allocated to separate plates, so differences between extract treatments and controls may reflect both treatment and plate effects. These effects could not be separated in the present design.

Inhibition zones were measured using a caliper. The measurements were performed with knowledge of the treatment concentrations; therefore, blinding was not implemented. The resolution of the caliper was not recorded and could not be verified. Together with the use of a single extract batch and plant source, these limitations restrict the generalizability of the findings to other extraction batches, collection events, or matoa plants. The results are therefore presented as preliminary and exploratory observations.

**Statistical Analysis**

Data are reported as individual replicate values and as means ± sample standard deviations (SD). All means and SDs presented in Tables 1 and 2 were recalculated from the individual replicate values.

The primary analysis was a one-way analysis of variance (ANOVA) across the four extract concentrations (15%, 20%, 25%, and 30% w/v), performed separately for each test organism at a significance level of $\alpha = 0.05$. Positive and negative controls were not included as concentration levels in the ANOVA because they were allocated to separate agar plates from the extract treatments. This arrangement introduced potential plate effects that could not be separated from treatment effects. Therefore, comparisons between extract treatments and controls were descriptive, and no Dunnett-type inferential comparison was performed.

Normality of ANOVA residuals was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using the Brown–Forsythe test. Because each concentration was represented by three technical replicates, both assumption tests had limited statistical power. Consequently, non-significant assumption tests were not interpreted as definitive confirmation that the assumptions were satisfied.

A linear trend contrast across the ordered extract concentrations was used to assess the presence of a linear concentration–response pattern. Effect sizes were expressed as eta-squared ($\eta^2$) and omega-squared ($\omega^2$). Ninety-five percent confidence intervals were reported for group means where calculated. The small number of technical replicates and the use of a single extract batch were considered when interpreting the statistical results. A non-significant result was not interpreted as evidence of equivalence between concentrations.

The experimental unit and replication structure are described above (Experimental Unit and Replication). Because the replicates were technical rather than independent biological replicates, the analyses characterize variation within the tested extract preparation and do not establish reproducibility across independent extraction batches or plant sources.

A Dunnett-type comparison against the controls was not adopted, because the negative control has zero variance, which violates the homogeneity assumption the procedure requires, and the controls are confounded with plate. Comparison against the controls is therefore descriptive only.

No post hoc test among concentrations was performed, because the omnibus test was not significant.

## RESULTS AND DISCUSSIONS

**Activity against *Staphylococcus epidermidis***

Given the small sample size (n = 3 per concentration), the study had limited power to detect differences between treatments. The estimated minimum detectable pairwise differences at 80% power were approximately 5.29 mm for the antibacterial assay and 5.11 mm for the antifungal assay (Table 4). Both values exceeded the observed ranges of the extract-treatment means, indicating that the non-significant findings should not be interpreted as evidence of equivalence between concentrations.

The recorded clear-zone measurements beyond the disc edges were 6.33 ± 2.08, 7.50 ± 1.50, 8.92 ± 2.74, and 9.33 ± 1.53 mm for extract concentrations of 15%, 20%, 25%, and 30% (w/v), respectively (Table 1). The negative control (10% DMSO) showed no visible inhibition beyond the disc edge, whereas the chloramphenicol control produced a mean measurement of 17.00 ± 2.00 mm.

The mean measurements increased across the tested extract concentrations; however, the one-way ANOVA did not detect a statistically significant difference among the four concentrations, $F(3, 8) = 1.373$, $p = 0.319$. Thus, the observed numerical increase should not be interpreted as evidence of a statistically significant concentration-dependent effect. The results are presented as preliminary observations under the conditions tested, rather than as a validated assessment of antimicrobial potency. No potency categories were assigned because the assay was not conducted using a standardized susceptibility-testing protocol and no applicable interpretive criteria were established for this crude extract.

a. Extract doses in parentheses are nominal values; the dissolved fraction of the crude extract was not analytically verified (see Preparation of Test Solutions).

**Table 1.** Clear-zone measurements beyond the disc edges for a 70% ethanol extract of *Pometia pinnata* fruit peel against *Staphylococcus epidermidis* ATCC 12228 on Mueller–Hinton agar after 24 h at 37 °C using the disc diffusion method (6-mm discs, 10 µL per disc). Positive control, chloramphenicol; negative control, 10% DMSO. Values represent three technical replicates.

| Treatment | Replicate 1 (mm) | Replicate 2 (mm) | Replicate 3 (mm) | Mean ± SD (mm) |
|---|---|---|---|---|
| Negative control (10% DMSO) | 0.00 | 0.00 | 0.00 | No zone |
| Positive control (chloramphenicol) | 19.00 | 15.00 | 17.00 | 17.00 ± 2.00 |
| 15% (1.5 mg/disc) | 4.00 | 7.00 | 8.00 | 6.33 ± 2.08 |
| 20% (2.0 mg/disc) | 6.00 | 7.50 | 9.00 | 7.50 ± 1.50 |
| 25% (2.5 mg/disc) | 6.75 | 8.00 | 12.00 | 8.92 ± 2.74 |
| 30% (3.0 mg/disc) | 8.00 | 9.00 | 11.00 | 9.33 ± 1.53 |

b. The negative control showed no visible inhibition beyond the disc edge.

c. The distance between adjacent discs was not measured or recorded. Potential effects of disc spacing and the separation of inhibition zones could therefore not be evaluated.

d. Means and standard deviations were calculated from the individual replicate values shown.

One-way ANOVA across the four concentrations was not significant, $F(3, 8) = 1.373$, $p = 0.319$. Residuals were consistent with normality (Shapiro–Wilk $W = 0.950$, $p = 0.642$) and variances with homogeneity (Brown–Forsythe $W = 0.184$, $p = 0.904$), subject to the power caveat given under Statistical Analysis. The effect size was $\eta^2 = 0.340$ with $\omega^2 = 0.085$, and the linear trend contrast did not reach significance, $t(8) = 1.990$, $p = 0.082$.

This non-significant result must not be read as evidence that the concentrations are equally effective. With $n = 3$ per group and a pooled within-group SD of 2.03 mm, the smallest pairwise difference this design could detect with 80% power is approximately 5.29 mm, which is larger than the entire 3.00 mm spread between the 15% and 30% means. The study is simply unable to resolve differences of the size plausibly present. Accordingly, no concentration is identified as optimal, and 30% is described only as having the highest recorded mean.

**Activity against *Trichophyton mentagrophytes***

The negative control produced no inhibition zone and ketoconazole produced a mean of 20.00 ± 2.65 mm. Recorded means for the extract were 14.00, 18.00, 16.67 and 15.00 mm at 15%, 20%, 25% and 30% respectively (Table 2). Unlike the antibacterial data, every antifungal value exceeds the 6 mm disc diameter, so these readings are internally consistent with the total-diameter convention, although the convention itself remains unconfirmed.

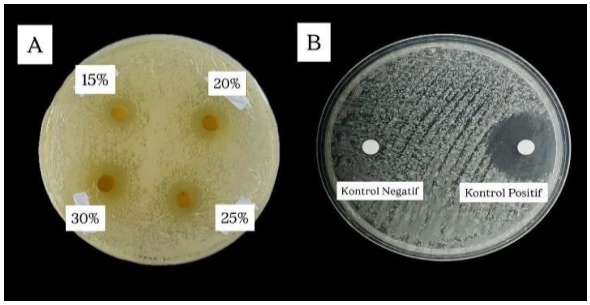


**Figure 1.** Disc diffusion against *Staphylococcus epidermidis* ATCC 12228 on Mueller–Hinton agar after 24 h at 37 °C. (A) One plate carrying all four extract concentrations: 15%, 20%, 25% and 30% (w/v) of a 70% ethanol extract of *Pometia pinnata* fruit peel, 10 µL per 6 mm disc. (B) A separate plate carrying the negative control (10% DMSO) and the positive control (chloramphenicol). Representative of three replicates.

One-way ANOVA was not significant, $F(3, 8) = 2.457$, $p = 0.138$, with residuals consistent with normality ($W = 0.973$, $p = 0.942$) and variances with homogeneity ($W = 0.815$, $p = 0.521$). The effect size was $\eta^2 = 0.479$ with $\omega^2 = 0.267$. The linear trend contrast was far from significant, $t(8) = 0.330$, $p = 0.750$, consistent with the absence of a monotone concentration–response relationship over this range.

The highest recorded mean occurred at 20% rather than 30%. This non-monotonic pattern is not interpreted as a causal effect of concentration. The differences concerned are not statistically distinguishable ($p = 0.138$) and the SD reaches 3.06 mm at 25%, so the ordering of the means is within the range of experimental variation and may reflect nothing more than noise. Solubility and diffusion limits of crude extracts in agar are real phenomena and are well described [12], but invoking them here

would be explaining a difference that has not been shown to exist. The honest reading is that no concentration–response relationship was detected across 15–30%, and that a higher concentration has not been shown to reduce antifungal activity.

A further caution applies to these data specifically. The photographic record (Figure 2) does not show four discrete, separately measurable circular zones. The growth-free areas merge into a single irregular central region spanning all four discs, and fungal growth is colonial and patchy rather than a confluent lawn. Assigning an individual zone diameter to each disc under these conditions is not straightforward, and the antifungal measurements should be regarded as provisional pending inspection of the full photographic record.

**Table 2.** Inhibition zone diameters recorded for a 70% ethanol extract of *Pometia pinnata* fruit peel against *Trichophyton mentagrophytes* ATCC 18748 on Sabouraud dextrose agar, read on day 7 after incubation at 25–28 °C, by disc diffusion (6 mm discs, 10 µL per disc). Positive control, ketoconazole; negative control, 10% DMSO. n = 3 technical replicates.

| Treatment | Replicate 1 (mm) | Replicate 2 (mm) | Replicate 3 (mm) | Mean ± SD (mm) |
|---|---|---|---|---|
| Negative control (10% DMSO) | 0.00 | 0.00 | 0.00 | No zone |
| Positive control (ketoconazole) | 21.00 | 22.00 | 17.00 | 20.00 ± 2.65 |
| 15% (1.5 mg/disc) | 14.00 | 16.00 | 12.00 | 14.00 ± 2.00 |
| 20% (2.0 mg/disc) | 17.00 | 19.00 | 18.00 | 18.00 ± 1.00 |
| 25% (2.5 mg/disc) | 16.00 | 20.00 | 14.00 | 16.67 ± 3.06 |
| 30% (3.0 mg/disc) | 15.00 | 16.00 | 14.00 | 15.00 ± 1.00 |

a. The source, formulation, concentration and application volume of ketoconazole were not determined; see Controls under Research Methods.

b. All antifungal values exceed the 6 mm disc diameter and are internally consistent with a total-diameter convention, which nonetheless remains unconfirmed.

c. Zone boundaries were assigned where clear areas around adjacent discs merged; see the text and Figure 2.

d. Means and standard deviations are calculated from the individual replicate values shown.

The antifungal findings should be interpreted in light of the methodological limitations of the assay. The fungal inoculum was prepared from colony material suspended in sterile physiological saline; however, the conidial concentration was not quantitatively determined, and a standardized conidial-counting procedure was not performed. The assay was conducted as preliminary agar-diffusion screening rather than according to the standardized CLSI M38 procedure for filamentous fungi. In addition, the reported results were based on a single observation time on day 7. The merged growth-free areas around adjacent discs further limited the reliability of assigning individual inhibition-zone measurements to each treatment. Future studies should use a standardized fungal inoculum, assess inhibition at appropriate observation times, and determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) using a broth microdilution approach.

**Table 3.** Comparison with published studies of *Pometia pinnata* extracts. Zone values are reproduced as given in each source; where a source does not state whether a value is net of the disc or well, this is noted, because that difference alone can account for several millimeters.

| Study | Plant part | Solvent | Method | Organism | Conc. | Zone (mm) | Limits to comparability |
|---|---|---|---|---|---|---|---|
| This study | Fruit peel | 70% ethanol | Disc, 6 mm, 10 µL | *S. epidermidis* ATCC 12228 | 15–30% (w/v) | 6.33–9.33 (mean) | Measurement convention unresolved; technical replicates only |
| This study | Fruit peel | 70% ethanol | Disc, 6 mm, 10 µL | *T. mentagrophytes* ATCC 18748 | 15–30% (w/v) | 14.00–18.00 (mean) | Single day-7 reading; merged zones on plate |
| Sulastri et al. [10] | Fruit peel | 96% ethanol | Well (*cetak lubang*) | *S. aureus* | 20, 40, 60% | 6.6, 8.3, 7.7 | Well not disc; different solvent and organism; non-monotonic response |

| Study | Plant part | Solvent | Method | Organism | Conc. | Zone (mm) | Limits to comparability |
|---|---|---|---|---|---|---|---|
| Sulistyorini et al. [11] | Fruit peel | Methanol | Disc | *S. mutans* | 25, 50, 75, 100% | 0, 0, 0.86, 1.63 | Values appear net of disc; title states ethanol while abstract states methanol |
| Sulistyorini et al. [11] | Fruit peel | n-Hexane | Disc | *S. mutans* | 25–100% | 0 at all | No activity detected |
| Pagarra et al. [8] | Leaves | 70% ethanol, 1:4 | Disc | *S. aureus* | 5, 7.5, 10% | 15.29 (7.5%), 18.22 (10%) | Different plant part; much lower concentrations |
| Pagarra et al. [8] | Leaves | 70% ethanol, 1:4 | Disc | *E. coli* | 5, 7.5, 10% | 10.87 (10%) | Different plant part and organism (Gram-negative) |
| Sidoretno and Gustari [9] | Leaves | Ethanol | Agar diffusion | *T. mentagrophytes* | 10, 20, 30% | 23.05, 24.86, 25.05 | Different plant part; monotonic rise, unlike the present study; ketoconazole control 28.56 mm |

**Table 4.** Statistical summary for the four extract concentrations. Controls were not entered as concentration levels (see Statistical Analysis).

| Quantity | *S. epidermidis* | *T. mentagrophytes* |
|---|---|---|
| One-way ANOVA across 15–30% | $F(3, 8) = 1.373$, $p = 0.319$ | $F(3, 8) = 2.457$, $p = 0.138$ |
| Shapiro–Wilk, residuals | $W = 0.950$, $p = 0.642$ | $W = 0.973$, $p = 0.942$ |
| Brown–Forsythe, variance homogeneity | $W = 0.184$, $p = 0.904$ | $W = 0.815$, $p = 0.521$ |
| Effect size $\eta^2$ ($\omega^2$) | 0.340 (0.085) | 0.479 (0.267) |
| Linear trend contrast | $t(8) = 1.990$, $p = 0.082$ | $t(8) = 0.330$, $p = 0.750$ |
| Pooled within-group SD | 2.03 mm | 1.96 mm |
| Smallest pairwise difference detectable at 80% power | ≈ 5.29 mm | ≈ 5.11 mm |

a. Assumption tests have very low power at n = 3; a non-significant result is weak evidence of conformity, not confirmation.

b. The detectable-difference row is given so that the non-significant ANOVA is not misread as evidence of equivalence.

c. If the plate is treated as a block (see Experimental Unit and Replication), a randomized complete block analysis applies in place of the one-way ANOVA reported here.

## Comparison with the Positive Controls

The positive controls produced observable inhibition zones under the experimental conditions used. However, their results cannot be compared quantitatively with those of the extract treatments as a direct measure of relative antimicrobial potency. The active antimicrobial concentrations delivered per disc were not verified, the control formulations differed from the crude extract solutions, and the recorded application volume of chloramphenicol was 30 µL per disc compared with 10 µL per disc for the extract treatments.

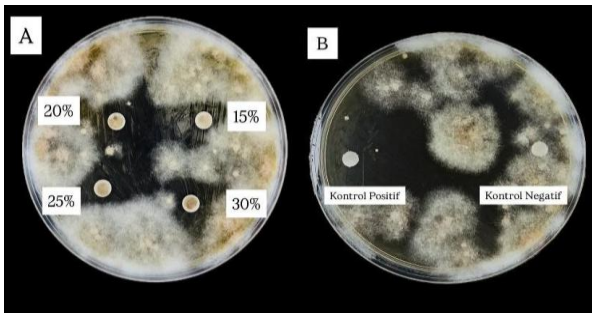


**Figure 2.** Disc diffusion against *Trichophyton mentagrophytes* ATCC 18748 on Sabouraud dextrose agar, photographed on day 7 after incubation at 25–28 °C. (A) One plate carrying all four extract concentrations: 15%, 20%, 25% and 30% (w/v), 10 µL per 6 mm disc. (B) A separate plate carrying the positive control (ketoconazole) and the negative control (10% DMSO). Representative of three replicates. Note that growth is colonial rather than a confluent lawn and that growth-free areas around adjacent discs merge; see the text.

In addition, the positive and negative controls were placed on a separate agar plate from the four extract treatments. Consequently, differences

between the extract and control measurements may reflect both treatment-related effects and plate-related variation. The negative control contained 10% DMSO; however, no separate control was included to assess the potential contribution of residual ethanol from the extraction process. No dedicated quality-control strain was included, and the assays were not conducted according to standardized CLSI susceptibility-testing procedures. The findings should therefore be interpreted as preliminary observations of inhibition under the conditions tested, rather than evidence of validated antimicrobial susceptibility or comparative potency.

## Comparison with Previous Studies of *Pometia pinnata*

Table 3 compares the present results with published studies of *P. pinnata*, with the limits of comparability stated for each.

Two patterns emerge. First, leaf extracts have consistently outperformed peel extracts. Sidoretno and Gustari reported zones of 23.05–25.05 mm against *T. mentagrophytes* at 10–30% [9] and Pagarra et al. reported 18.22 mm against *S. aureus* at 10% [8], both above the present peel results at higher concentrations. Their leaf extract produced larger zones at lower concentrations and showed a monotonic rise across 10–30%, whereas the present peel extract produced smaller zones with no detectable trend. The findings differ, and the difference is informative about plant part.

Second, the present antibacterial results sit within the weak range established for peel extracts. Sulistyorini et al. recorded zones of only 0.86–1.63 mm against *S. mutans* even at 75–100% [11], values that are evidently net of the disc. If the present antibacterial readings are total diameters, the corresponding net zones of 0.33–3.33 mm are of the same order. Two independent studies of matoa fruit peel therefore converge on weak antibacterial activity against Gram-positive cocci, which is the most defensible conclusion the present data support.

Direct comparison remains limited throughout. The studies differ in extraction solvent (70%, 96% ethanol, methanol, n-hexane), in diffusion format (disc versus well), in test organism, and in whether zone values are reported net or total. Differences in zone diameter between these studies cannot be attributed to differences in antimicrobial activity alone [12,13].

## Candidate Constituents and the Limits of Mechanistic Inference

No phytochemical screening was performed on the extract tested here, so no constituent can be identified as responsible for the observed activity. Qualitative screening of dried matoa fruit peel has detected flavonoids, tannins and saponins, and has specifically failed to detect alkaloids, quinones, steroids and triterpenoids [7]. Candidate constituents are therefore restricted to flavonoids, tannins and saponins. Mechanisms by which flavonoids, tannins and saponins act on bacteria have been reviewed [14], but those are literature-derived hypotheses, not findings of this study.

Two mechanistic studies often cited for plant antimicrobials do not transfer to this material. Wang et al. examined a *Rhizoma Coptidis* extract against *S. epidermidis* ATCC 35984 [15], a different plant and a biofilm-forming strain; the present work used ATCC 12228, which lacks the *ica* operon [4]. Yang et al. examined Huangqin decoction against *T. mentagrophytes* and determined a minimum inhibitory concentration (MIC) of 3.13 mg $mL^{-1}$ by broth microdilution [16]; that is a different preparation, and the pathways they identified cannot be assigned to matoa peel. Both indicate the kind of evidence that would be required here.

## Limitations

The limitations below are those that actually apply to this work, and several of them constrain the conclusions materially rather than marginally.

(1) The measurement convention for inhibition zones is unresolved, and one antibacterial value is smaller than the disc. Until this is settled the antibacterial results cannot be interpreted with confidence (see Measurement of Inhibition Zones).

(2) Replication is technical, not biological. One tree, one collection and one extraction batch mean the results describe a single extract preparation and cannot be generalized to matoa fruit peel from other trees, locations or seasons.

(3) The four concentrations shared a plate, so the disc is not an independent experimental unit and the controls are confounded with plate.

(4) Statistical power is very low. The design cannot detect pairwise differences smaller than roughly 5 mm, so non-significant results are uninformative about equivalence.

(5) Botanical authentication, extract yield and chemical characterization are not available.

(6) Complete dissolution of the crude extract was not verified, so delivered dose may be below the nominal dose.

(7) The positive controls are inadequately specified and not dose-matched to the extract, limiting them to a qualitative indication that the assay functioned.

(8) One strain per species was tested. ATCC 12228 is a non-biofilm-forming commensal isolate [4] and no clinical or biofilm-forming isolate was examined.

(9) Agar diffusion has known limitations for crude plant extracts, whose less polar constituents diffuse poorly through an aqueous agar matrix; zone diameter consequently reflects diffusion as well as activity, and results are not readily comparable between laboratories [12].

(10) No MIC, minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was determined, so the data cannot distinguish inhibition from killing or establish a potency value [13,17].

(11) The antifungal assay was read at a single time point, day 7, and the choice of that endpoint is not justified by a cited method.

(12) Cytotoxicity, stability and skin-penetration data are absent.

(13) The assays were not performed according to CLSI M02 or M38 [18,19], and no quality-control strain was included; no claim of conformity with any standard is made. Interpretive breakpoints of the kind published in CLSI M100 [20] apply to defined antimicrobial agents, not to crude plant extracts, so the zone diameters reported here cannot be translated into a susceptibility category or any potency classification.

These are *in vitro* screening results on agar. They cannot be extrapolated to the treatment of skin infection *in vivo*.

## CONCLUSIONS

A 70% ethanol extract of matoa fruit peel produced measurable inhibition zones against *Trichophyton mentagrophytes* ATCC 18748 at 15–30% (w/v), with recorded means of 14.00–18.00 mm, and against *Staphylococcus epidermidis* ATCC 12228 at 20–30%. The 15% result against *S. epidermidis* is equivocal, and activity at every tested concentration against this organism is not established, because the measurement convention could not be reconciled with values recorded at or below the 6 mm disc diameter. No concentration–response relationship was detected for either organism, and no concentration can be designated optimal; the apparent differences between concentrations lie within the resolution of a three-replicate design.

The principal constraints on these conclusions are the unresolved measurement convention, the technical rather than biological nature of the replication, and the inadequately specified positive controls. The work should be read as a preliminary screen of a single extract preparation.

Establishing whether matoa fruit peel is a useful antimicrobial lead requires independent biological replication across trees and extraction batches, MIC, MBC and MFC determination by broth microdilution, phytochemical profiling of the tested extract, testing against clinical and biofilm-forming isolates, and cytotoxicity assessment before any dermatological application is contemplated.

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## CONFLICT OF INTEREST

The authors have no financial conflicts of interest to declare.

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## AUTHOR CONTRIBUTIONS

N.I.A.: Investigation, Formal analysis, Writing – original draft. R.: Conceptualization, Methodology, Supervision, Data curation, Writing – review & editing. B.K.: Conceptualization, Methodology, Supervision, Visualization, Writing – review & editing. Y.H.: Validation, Resources, Writing – review & editing. A.A.: Validation, Supervision, Writing – review & editing. All authors read and approved this paper.

## DECLARATION OF GENERATIVE AI USE

AI-assisted language tools were used to improve clarity, grammar, and readability of the manuscript. The authors carefully reviewed and edited the content as needed and take full responsibility for this paper.

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