Preliminary Analysis Report on the Effect of Aloe arborescens Compounds on Candida albicans Morphogenesis

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Introduction

Fungal infections pose a significant challenge to global healthcare, with Candida albicans being one of the most prevalent opportunistic fungal pathogens. C. albicans is commonly found as part of the human microbiota but can become pathogenic under certain conditions, leading to infections ranging from superficial mucosal infections to life-threatening systemic diseases, especially in immunocompromised individuals. The rising resistance to conventional antifungal drugs has intensified the demand for alternative, effective treatments. This has spurred interest in natural compounds, particularly those derived from plants known for their medicinal properties.

Aloe arborescens, a species of the Aloe genus, has been traditionally used for its medicinal qualities, including antimicrobial effects. Preliminary studies suggest that compounds isolated from Aloe arborescens may have antifungal properties that could inhibit the morphogenesis of C. albicans, thereby preventing its pathogenic transition. Morphogenesis in C. albicans, specifically the formation of hyphae, is a key factor in its virulence. This study investigates whether compounds isolated from Aloe arborescens can effectively inhibit hyphal and other morphological formations in C. albicans.

This report provides a preliminary, descriptive analysis of the effects of seven treatments derived from Aloe arborescens on C. albicans morphogenesis. These treatments include isolated compounds and commercially sourced variants, individually and in combination. By assessing changes in morphological responses, this study aims to identify potential inhibitory effects that could pave the way for developing novel antifungal therapies. The insights gained here will guide future inferential analyses to quantify the efficacy of these treatments, ultimately contributing to antifungal research and alternative medicine.

Data Summary

The dataset comprises morphogenesis counts for Candida albicans cells exposed to seven treatment conditions. Each treatment included 200 cells across five replicates, resulting in 1,000 observations per treatment. The following **morphological responses** were recorded for each cell:

No Morphology Change: No formation of germ tubes, pseudohyphae, or buds.
Germ Tubes Only (GT Only): Cells exhibiting only germ tube formation.
Pseudohypha Only (PH Only): Cells showing only pseudohypha formation.
Buds Only: Cells with budding morphology.
Combinations: Cells displaying combinations of the above responses (e.g., GT + PH).

Treatment Types Analyzed

No Treatment (Control)
Filtered Whole Aloe arborescens
Extract Compound A (Isolated from Aloe arborescens)
Compound A-SA (Commercial Source)
Compound B (Isolated from Aloe arborescens)
Compound B-SA (Commercial Source)
Combination of Compounds A and B

Descriptive Analysis

Data Cleaning

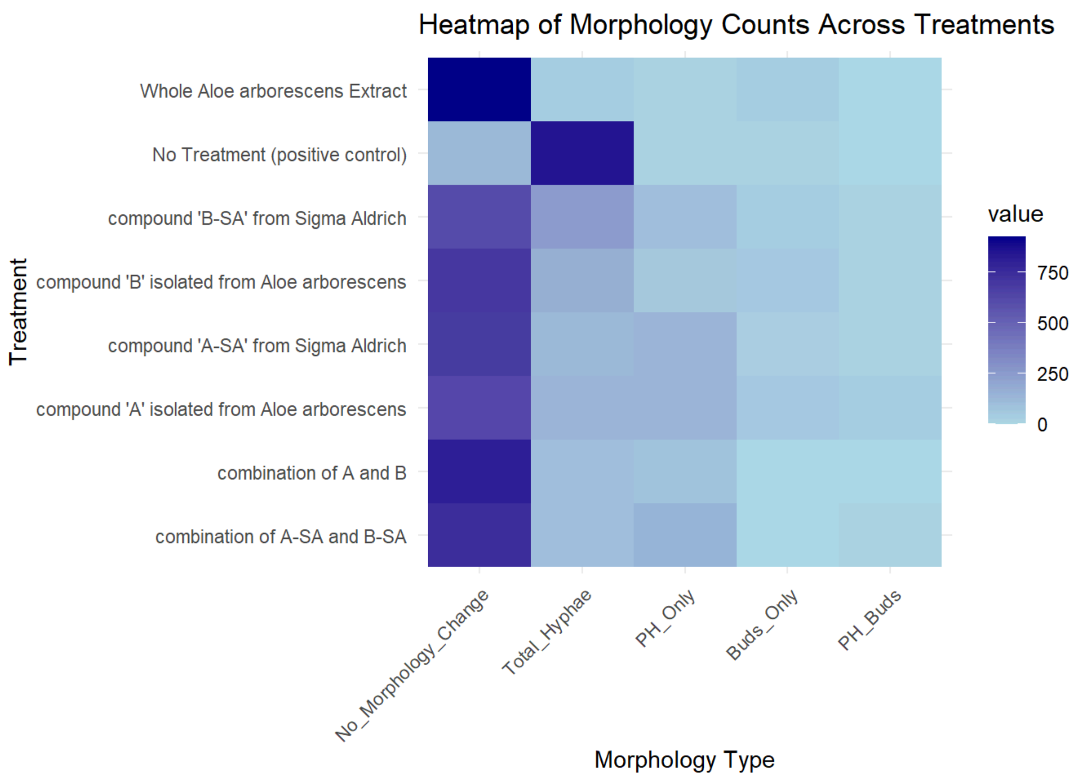
The column names in the original document had to be renamed to facilitate smoother analysis. The first column, which typically represents the treatments was converted to a factor variable. We also calculate "Total Hyphae" as the sum of all variables with Germ Tubes. That is,

$$\mathbf{Total\_Hyphae} = \mathbf{GT\_Only} + \mathbf{GT\_PH} + \mathbf{GT\_Buds} + \mathbf{Multiple\_GTs}$$

We then make the following plots to visualize the distributions of the dataset.

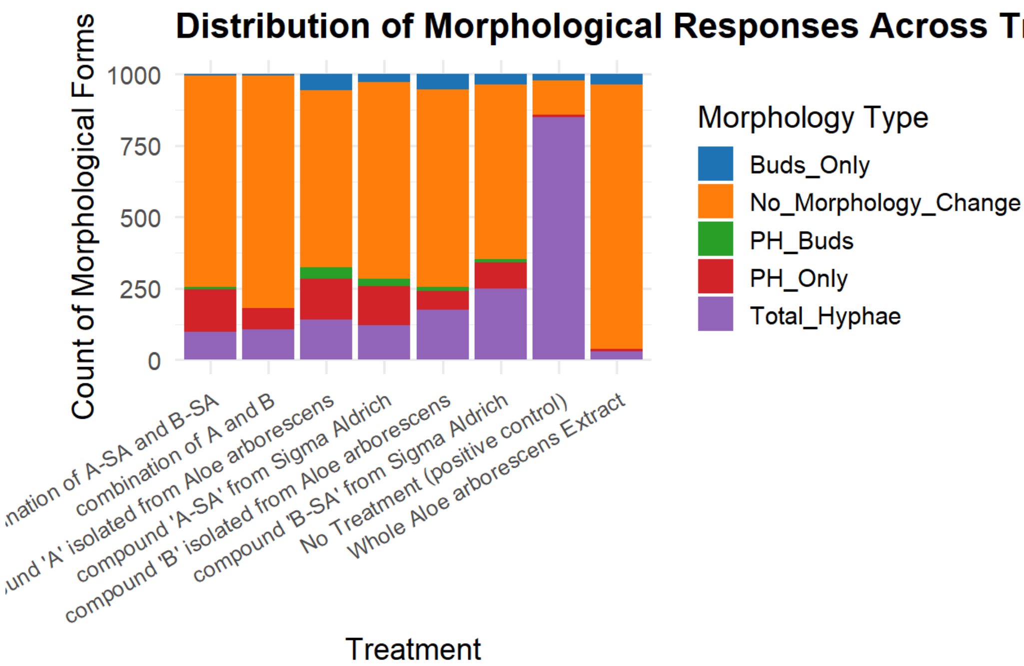
1. **Heatmap of Morphology Counts**

- **Heatmap Visualization**: A heatmap was created to highlight the counts of each morphological form across treatments. Darker shades represent higher counts, allowing a quick assessment of which treatments most effectively inhibit specific morphology types.
  - **Observations**:
    - **Positive Control** had high counts for **Total_Hyphae** cell, while **Whole Aloe Extract** and **Compound A** showed reduced germ tube formation, indicated by lighter colors in the **GT Only** column.
    - The **combination treatment of A and B** and **combination treatment of A-SA and B-SA** resulted in a marked increase in "No Morphology Change" counts, suggesting an effective suppression of fungal morphogenesis.



2. **Morphological Distribution Across Treatments**

- **Stacked Bar Plot**: A stacked bar plot was created to visualize the distribution of each morphological type within the seven treatment groups. This visualization highlights that the positive control (no treatment) shows a high prevalence of Total Hyphae formation, suggesting that untreated cells primarily form germ tubes.
  - **Observations:**
    - **No Treatment (Positive Control)** predominantly showed "Total Hyphae" morphology, suggesting natural germ tube formation when *Candida albicans* is unexposed to any compounds.
    - **Filtered Aloe Extract** and **Compound A** treatments exhibited reduced "Total Hyphae" counts compared to the control, suggesting a potential inhibitory effect on germ tube formation.
    - **Combination Treatment (A and B)** and "**Combination Treatment (A-SA and B-SA)**" displayed an increase in "No Morphology Change" compared to single compounds, indicating a possible synergistic effect in reducing fungal morphogenesis.



Summary statistics for each morphology type are presented in table titled “Summary Statistics of Morphological Responses by Treatment” with metrics mean, median, and standard deviation across replicates for each treatment.

Summary Statistics of Morphological Responses by Treatment

Treatment	No Morphology Change Mean	SD	Median	Total Hyphae Mean	SD	Median	PH Only Mean	SD	Median	Buds Only Mean	SD	Median	PH_Buds Mean	SD	Median
combination of A-SA and B-SA	148.2	13.14	153	19.4	4.56	18	30.0	9.33	26	1.0	1.41	0	1.4	2.19	0
combination of A and B	162.8	12.09	163	21.4	3.29	22	14.8	11.88	13	0.8	1.30	0	0.2	0.45	0
compound ‘A’ isolated from Aloe arborescens	124.4	12.82	124	28.2	9.98	27	28.4	3.85	29	11.0	4.36	10	8.0	2.24	9
compound ‘A-SA’ from Sigma Aldrich	137.8	10.33	133	24.0	4.64	27	27.6	3.29	27	5.6	4.72	6	5.0	2.74	5
compound ‘B’ isolated from Aloe arborescens	138.2	11.30	136	34.8	7.95	36	13.4	4.51	13	10.6	4.56	10	3.0	1.87	3
compound ‘B-SA’ from Sigma Aldrich	122.8	15.29	124	49.8	9.42	53	18.6	9.86	15	7.0	2.24	7	1.8	1.30	1

Treatment	No Morphology Change Mean	SD	Median	Total Hyphae Mean	SD	Median	PH Only Mean	SD	Median	Buds Only Mean	SD	Median	PH_Buds Mean	SD	Median
No Treatment (positive control)	24.0	7.11	21	170.0	6.82	173	1.8	1.30	2	4.2	2.86	4	0.0	0.00	0
Whole Aloe arborescens Extract	185.4	7.23	187	6.0	2.35	7	1.4	1.95	0	7.2	6.46	5	0.0	0.00	0

Statistical Analysis Plan

The experiment follows a one-factor design with eight treatment groups, including a control group. Each treatment represents a different exposure condition for *Candida albicans*, with the primary objective being the evaluation of specific, pre-planned comparisons of interest rather than an exhaustive comparison across all treatment levels. This targeted approach emphasizes planned (a priori) contrasts to minimize Type I error, ensuring that we focus only on the research goals without unnecessary testing.

The response variable, **total hyphae formation**, is a count of germ tube (GT) formations per treatment replicate. In our dataset, we realize this count deviates from normality, making standard normal-theory approaches like ANOVA unsuitable.

A Quasi-Poisson Regression model is identified as the suitable for this analysis. The quasi-Poisson model is suitable for this analysis because of the nature of the response variable, count data (the number of germ tubes formed in *Candida albicans* cells). The model accounts for this mild overdispersion by introducing an overdispersion parameter (1.349444), which is the ratio that adjusts the standard errors without altering the expected mean structure. This adjustment provides more accurate p-values and confidence intervals. Also, in quasi-Poisson, the interpretation remains straightforward: each treatment's effect can be expressed as a rate ratio compared to the reference group, which is very straightforward for hypothesis testing. We employ a generalized linear model (GLM) using the quasi-Poisson distribution, with the following structure:

Model Formula:

$$\mathbf{log(Total\ Hyphae)} = \alpha + \beta \cdot \mathbf{Treatment} + log(200)$$

where:

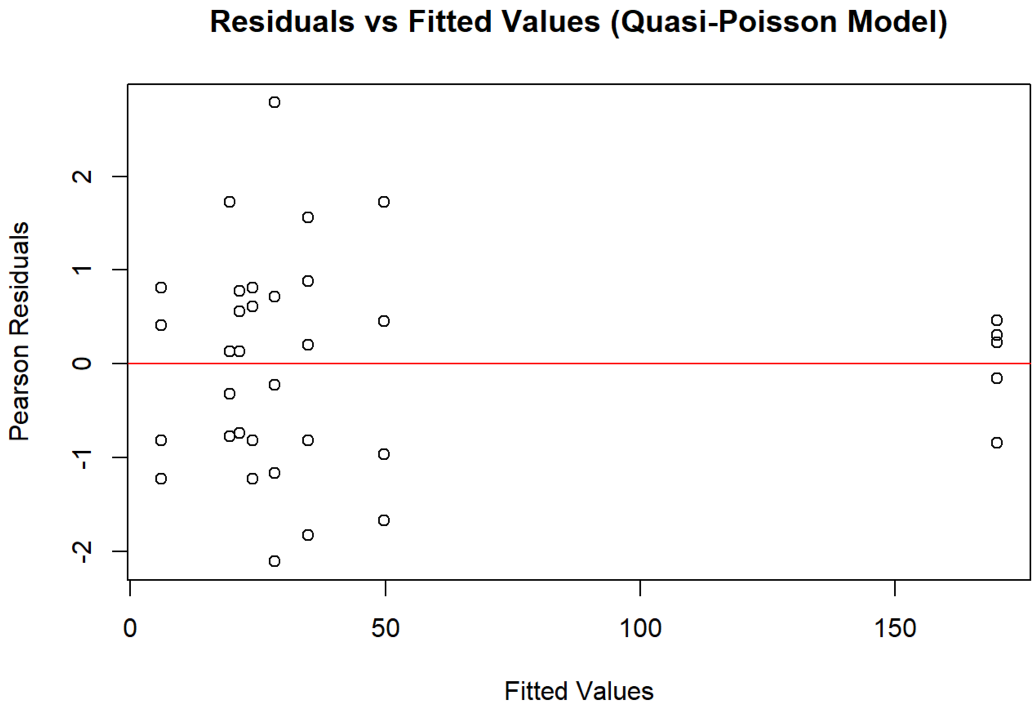
α is the intercept representing the log rate of hyphae formation for the control group.

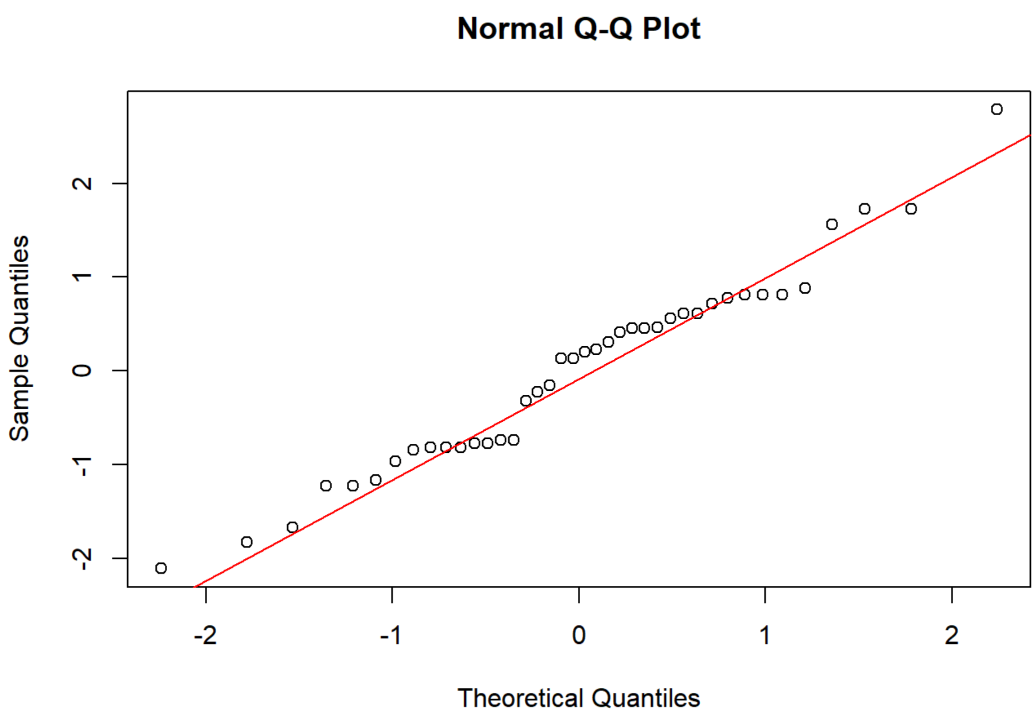
$\beta \cdot \mathbf{Treatment}$ represents the effect of each treatment on the log rate of hyphae formation.

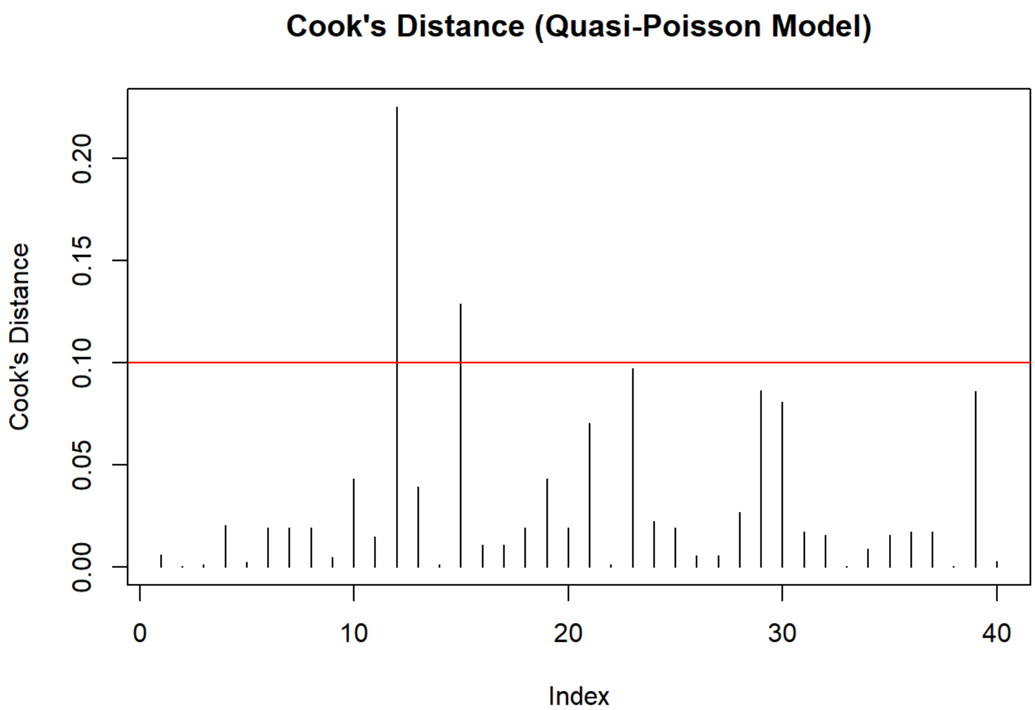
$log(200)$ serves as an offset term, adjusting for the cell count per replicate (200 cells per replicate).

Model Diagnostics Checks

The dispersion statistic, as stated earlier, was given as 1.349444. This value, neither too low nor high, suggests the quasi-Poisson model is suitable. The plot of residuals against fitted values does not reveal any obvious patterns. The normal Q-Q plot shows a bit of deviation from the line, which indicate our residuals are not normally distributed, which was expected as this is common in count data models. Although quasi-Poisson models don't use likelihood-based methods, a Chi-square goodness-of-fit test is still useful to evaluate the overall fit. With a non-significant p-value of 0.0896, the model fits the data adequately. There are however a few observations above the red line in the Cook's Distance plot.


Residuals vs Fitted Values (Quasi-Poisson Model)
Pearson Residuals
Fitted Values
-2
-1
0
1
2
0
50
100
150


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles
-2
-1
0
1
2



Results & Analysis

The treatments in the original dataset were given new labels for easy identification as original names were overly complex.

Research Hypothesis 1: Does exposure to each of the seven treatments result in statistically significant reductions in hyphae formation compared to non-exposed cells?

For this hypothesis, we use the **Dunnett-style multiple comparison test** to check whether each of the seven treatments leads to a statistically significant reduction in hyphae formation in *Candida albicans* compared to the control (non-exposed cells). Each treatment has a p-value < 0.0001, indicating that the reductions in hyphae formation are highly statistically significant for all seven treatments when compared to the control.
The Dunnett-adjusted p-values control for the family-wise error rate across the seven comparisons, confirming that the observed reductions are unlikely to be due to chance.
The estimates provided are on the log scale. Exponentiating these values gives the rate ratios of hyphae formation for each treatment relative to the control. Since all estimates are negative, exponentiating them will yield rate ratios less than 1, meaning that each treatment reduces hyphae formation relative to the control. For example: Whole Aloe Extract has an estimate of -3.34, which suggests a large reduction. $exp(-3.34) \approx 0.035$, indicating that the total hyphae formation rate under this treatment is approximately 3.5% of the control rate. The treatments vary in their effectiveness, with the Whole Aloe Extract and combinations of compounds (A and B or A-SA and B-SA) showing the largest reductions in hyphae formation relative to the control. Isolated compounds (e.g., Compound B-SA) also significantly reduce hyphae formation but to a lesser degree than the whole extract or combinations.

```
##  contrast              estimate     SE  df z.ratio p.value
##  Whole - Control          -3.34 0.2158 Inf -15.496  <.0001
##  A - Control              -1.80 0.1056 Inf -17.007  <.0001
##  (A-SA) - Control         -1.96 0.1133 Inf -17.282  <.0001
##  B - Control              -1.59 0.0967 Inf -16.410  <.0001
##  (B-SA) - Control         -1.23 0.0837 Inf -14.667  <.0001
##  A_B - Control            -2.07 0.1192 Inf -17.392  <.0001
##  (A-SA_B-SA) - Control    -2.17 0.1245 Inf -17.434  <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: dunnettx method for 7 tests
```

Research Question 2: Does treatment with Compound A alone result in greater reductions in hyphae formation compared to treatment with Compound B alone?

We're interested in the average effect of Compound A treatments (A and A-SA) versus Compound B treatments (B and B-SA). Rather than comparing each treatment individually, the contrast allows us to aggregate and compare the average log rate of germ tube formation for the two groups. Below is how contrasts were setup mathematically;

$$\text{Contrast}_{\text{A vs B}} = \left(\frac{\beta_{\text{A}} + \beta_{\text{A-SA}}}{2}\right) - \left(\frac{\beta_{\text{B}} + \beta_{\text{B-SA}}}{2}\right)$$

where β_{A} and $\beta_{\text{A-SA}}$ represent the log rates of germ tube formation for Compound A treatments, and β_{B} and $\beta_{\text{B-SA}}$ represent the log rates for Compound B treatments.
The p-value < 0.0001 is highly significant, indicating that this reduction in germ tube formation due to A and A-SA treatments is statistically significant compared to B and B-SA treatments.
The z-ratio of -5.100 (a large magnitude) further reinforces that this difference is meaningful and not due to random variation.
The contrast estimate is -0.47 on the log scale. Exponentiating this estimate gives a rate ratio of approximately $exp(-0.47) \approx 0.625$, which implies that the rate of germ tube formation in *Candida albicans* cells exposed to Compound A treatments is 63% of the rate observed in cells exposed to Compound B treatments.

```
##  contrast estimate     SE  df z.ratio p.value
##  A_vs_B      -0.47 0.0922 Inf  -5.100  <.0001
##
## Results are given on the log (not the response) scale.
```

Research Question 3: Does the 0.2-micron filtered Aloe arborescens extract treatment yield the greatest reduction in hyphae formation compared to the other treatments?

All six comparisons have highly significant p-values (p < 0.0001), confirming that each of the six treatments has a higher rate of germ tube formation than the Whole Aloe arborescens extract treatment.
Each estimate is positive, indicating that the Whole Aloe arborescens extract results in a lower log rate of germ tube formation compared to each of these six treatments.

```
##  contrast            estimate    SE  df z.ratio p.value
##  A - Whole               1.55 0.234 Inf   6.626  <.0001
##  (A-SA) - Whole          1.39 0.237 Inf   5.846  <.0001
##  B - Whole               1.76 0.230 Inf   7.655  <.0001
##  (B-SA) - Whole          2.12 0.225 Inf   9.426  <.0001
##  A_B - Whole             1.27 0.240 Inf   5.299  <.0001
##  (A-SA_B-SA) - Whole     1.17 0.243 Inf   4.836  <.0001
##
## Results are given on the log (not the response) scale.
```

The results show that the Whole Aloe arborescens extract yields the greatest reduction in germ tube formation compared to each of the other six treatments (A, A-SA, B, B-SA, A+B, A-SA+B-SA). This conclusion is supported by the positive and significant estimates in all six comparisons, indicating that the Whole extract is the most effective among the treatments tested.

Conclusion

This study investigated the effects of compounds derived from *Aloe arborescens* on the morphogenesis of *Candida albicans*, specifically focusing on the reduction of hyphae and germ tube formation—a critical factor in fungal pathogenicity. The hypotheses examined whether various treatments, including isolated compounds, commercially available equivalents, and whole extract, significantly inhibited germ tube formation compared to non-exposed cells.

Efficacy of Whole Aloe arborescens Extract:
The results indicated that the Whole Aloe arborescens extract was the most effective treatment in reducing hyphae formation compared to all other treatments, demonstrating a substantial and statistically significant reduction. This finding highlights the potential of the whole extract as a powerful antifungal agent that could be explored further for therapeutic applications.

Comparison Between Compound A and Compound B Treatments:
Treatments involving Compound A (both isolated and commercially sourced) resulted in a significantly greater reduction in germ tube formation compared to Compound B treatments. This suggests that specific active components within Compound A may have stronger antifungal properties, possibly due to their unique composition or potency in inhibiting morphogenesis in Candida albicans.

Relative Effectiveness of All Treatments:
All treatments, including isolated compounds and combinations, showed statistically significant reductions in hyphae formation when compared to the control group (non-exposed cells), affirming that Aloe arborescens derivatives generally possess antifungal properties.

Among these, however, the whole extract and certain combinations were notably more effective, suggesting synergistic effects that may enhance antifungal efficacy.

These findings contribute to the broader field of natural antifungal agents, which is particularly relevant given the global increase in resistance to conventional antifungal treatments. The use of Aloe arborescens compounds, especially the whole extract, could provide a basis for developing new antifungal formulations that are both safe and effective for treating fungal infections in clinical settings.