Evaluation of Acemannan as a Potential Immunoenhancer for Phagocytic and Killing Activities of Macrophages against *Candidozyma* (*Candida*) *auris*

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01 Introduction

Figure 1: *C. auris* grown on Sabouraud dextrose agar (SDA), 1 day, at 37 °C

What is *C. auris*?
- First identified in the ear canal in 2009
- Opportunistic yeast pathogen

Why so scary?
- Spreads easily in hospitals
- Affects immunocompromised patients
- Can cause life-threatening infections
- **Multidrug-resistant** - hard to treat

Problem?
- If no effective drugs, the immunocompromised are at risk!

How about boosting **host immunity** to help control *C. auris* infection?

Previous study:
Acemannan boosted macrophage killing of *C. albicans* from 5% to 98% in 1 hour (Stuart et al., 1997)

Hence, this study targets:
Acemannan (AC) from ***Aloe vera*** & **macrophage (MΦ)**

Figure 2: *Aloe vera* plant

Acemannan
- Acetylated long-chain mannan
- Potential immunostimulant

02 Aims & Objectives

Research Question
- **Could AC boost MΦ phagocytosis and killing activity against *C. auris*?**

Aims
- To assess the potential immune-enhancing functions of AC towards MΦ against *C. auris*

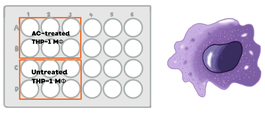
Objectives
- To compare MΦ phagocytosis and killing of *C. auris* between the treatment group (MΦ with AC stimulation) and the control group (MΦ without AC stimulation)

03 Materials & Methods

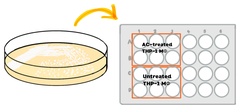
- THP-1 monocytes were differentiated into MΦ with phorbol 12-myristate 13-acetate (PMA)
- AC cytotoxicity towards THP-1 MΦ and *C. auris* was assessed by methyl thiazolyl tetrazolium (MTT) and EUCAST minimum inhibitory concentration (MIC) assays, respectively
- The infection assays (phagocytosis & killing activity) were performed in duplicate: an unpaired two-tailed t-test was conducted using GraphPad Prism 10

Key Materials
- Clade II *C. auris* (CBS 10913^T), first ear canal isolate from Japan
- THP-1 monocytic cell line
- AC (10^{-4} mg/mL as testing concentration)
- RPMI 1640 medium
- Phosphate-buffered saline (PBS)
- PMA
- SDA
- Sabouraud dextrose broth (SDB) with 0.01% triton X-100
- 24-well plate

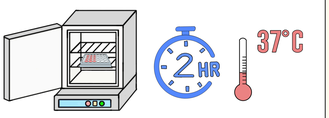
1) Seeding of AC-treated MΦ + untreated MΦ in 24-well plate



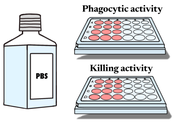
2) *C. auris* infection to MΦ



3) 2-hour incubation to allow MΦ ingesting *C. auris*

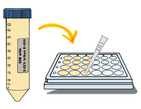


4) PBS wash to remove extracellular yeasts

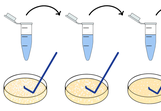


Killing activity:
Further **22 hours** of incubation after PBS wash to allow MΦ killing *C. auris*

5) MΦ lysis to collect intracellular yeasts



6) Ten-fold serial dilution of lysates & spreading on SDAs



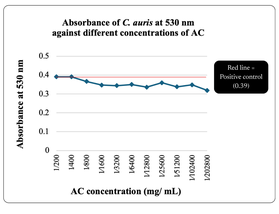
7) CFUs counting after 2 days of incubation

Figure 3: Flowchart of phagocytic and killing activities

04 Results

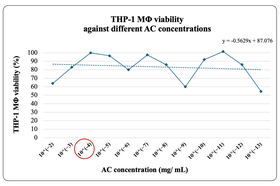
Based on MIC and MTT assay results, 10^{-4} mg/mL of AC showed no cytotoxicity towards THP-1 MΦ and *C. auris*

MIC assay:



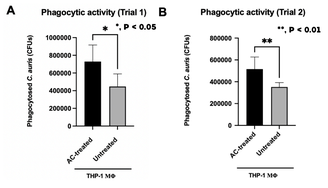
Highest tested concentration of AC (1/200 mg/ mL) had no inhibitory effect on *C. auris*

MTT assay:



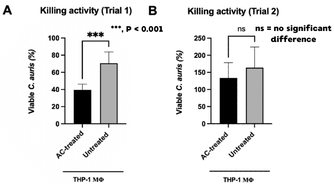
At concentrations ≤ 10^{-4} mg/mL, AC showed no significant cytotoxic effects on THP-1 MΦ

Phagocytic activity: consistent results in trials 1 and 2 = AC-treated MΦ ingested more yeasts



- Upon AC treatment, MΦ secreted more pro-inflammatory cytokines (Kumar & Kumar, 2019)
- AC may upregulate MΦ surface pattern recognition receptors that recognise pathogen-associated molecular pattern of *C. auris*
- Facilitate pseudopod formation & subsequent engulfment
- Enhance phagocytosis

Killing activity: inconsistent results in trials 1 and 2



Trial 1: AC-treated MΦ killed more yeasts	Trial 2: No significant difference
• Upon AC treatment, MΦ secreted more nitric oxide (NO) (Kumar & Kumar, 2019) • With increased NO levels, more intracellular yeasts are killed	Reason 1 Phagocytosed yeasts could induce oxidative stress response, which neutralised MΦ-secreted reactive oxygen species (Miramón et al., 2023) Reason 2 Technical error- insufficient PBS washing

05 Conclusion

Phagocytic activity
- **AC could enhance MΦ-mediated phagocytosis of *C. auris***
- **Acetyl groups in the compound may contribute**

Killing activity
- **Inconclusive, may be due to human error**
- **Reperforming the assay is warranted**

Outlook
- Highlights the possibility of a novel treatment approach for the susceptible hosts to combat deadly *C. auris*
- Herbal compound complementing with effective antifungals might have a synergistic effect
  - Concurrently boosts host immunity and attacks the yeast pathogen
  - Improves patient outcome, minimises drug resistance

Acknowledgements

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