

INTRODUCTION

Aim: This research endeavour will determine and evaluate the mechanism of action of a modified coconut oil that has previously shown promising antimicrobial activity against the opportunistic infection, *T. rubrum*.

Background: Dermatophyte disorders are among humanity's most persistent parasitic associates, and by now have survived several generations of therapeutic regimens. One concern in particular is the clinical difficulty of obtaining permanent treatments for fungal infections in the distal and lateral subungual space of the human nail. Another current worry remains the limited number of antifungal treatments available on the market, and with the widespread use of a small selection of such agents, there is a fear of a potential rise in antifungal resistance. These issues highlight the demand for novel antifungal therapeutic agents and with scaffolds offered by nature becoming increasing popular, they might just be the perfect solution to combat the concerns. One such offering is under development by a research group in TUS midlands.

HOW?

What effect has modification on the coconut oil?

What is the modified oil doing to the fungus?

Is the modified oil fungistatic or fungicidal?

WHY?

The nature of the ingredients activated by the modification procedure

Their characteristics and properties that allow it to invade the fungus.

Fungal characteristics that may make it susceptible.

METHODS

Clinical isolates of *T. rubrum* will be cultivated, isolated and quantified by microbial procedures. A modified coconut oil (MCO) formulation will be prepared according to a proprietary procedure and the components of the formulation will be determined by GC-MS analysis supported by vibrational spectroscopy in order to elucidate the predominant antimicrobial properties. *T. rubrum* will then be challenged with the modified oil through *in vitro* bioassays and scanning electron microscopy or transmission electron microscopy will be used to reveal the morphological effects on the fungus.

➤ Methods of analysis that have already been carried out include:

- Bomb calorimetry** on the modified oil along with other oils of interest to allow comparisons to be made relating to energy content.
- Thin layer chromatography** has also been preformed using the reference oil and other oils of interest to allow comparisons to be made, however, so far this method seems unsuccessful.
- Disc diffusion assay** using MCO and virgin coconut oil as a control against *C. albicans*
- Validation tests** on the MCO such as **Acid value test** and **Infrared spectroscopy**.

Exploratory runs by GC machine have been performed. Solvents, columns and assays have now been chosen to carry out work on the MCO.

METHODS CONT.

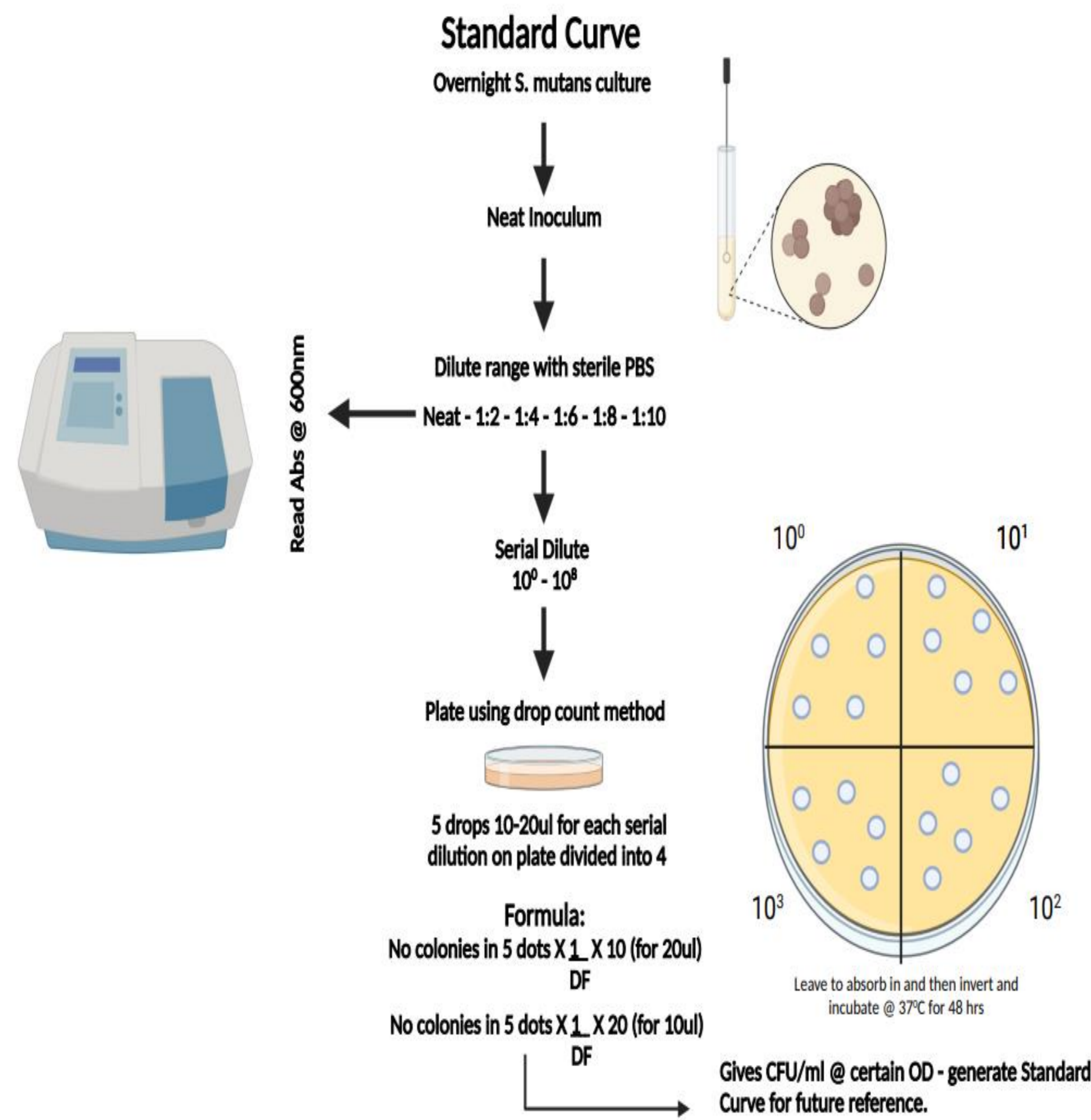


Figure 1 Standard curve procedure to obtain colony forming units per ml. Image created using biorender.

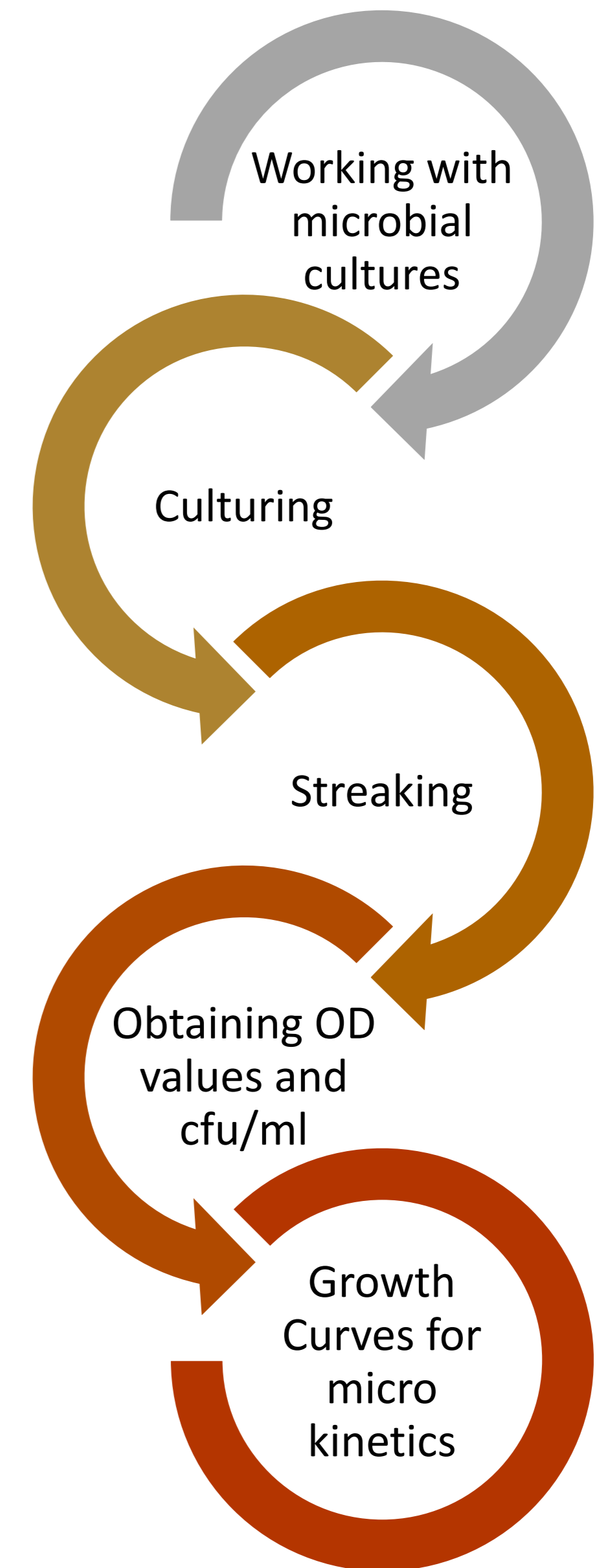


Figure 2 Flow diagram layout of initial methods carried out for this research

EXPECTED RESULTS

The hope of this research project is to uncover the mechanism of action by establishing the low concentration ingredients in the oil that are attributing to the antifungal action. By carefully determining the nature of these entities, the reproducibility of their occurrence and their stability in the oil, it will allow an understanding of the heretofore unexplained antifungal action. Finally, one expects to identify what makes the *T. rubrum* susceptible to this promising antifungal agent.

FUTURE DIRECTION

- Culture and quantify *T. rubrum*
- Total organic carbon analysis.
- Compositional analysis using GC-MS supported by vibrational spectroscopy.
- Bioassay *in-vitro*.
- Microscopy analysis using SEM and TEM.

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RESULT EXAMPLES

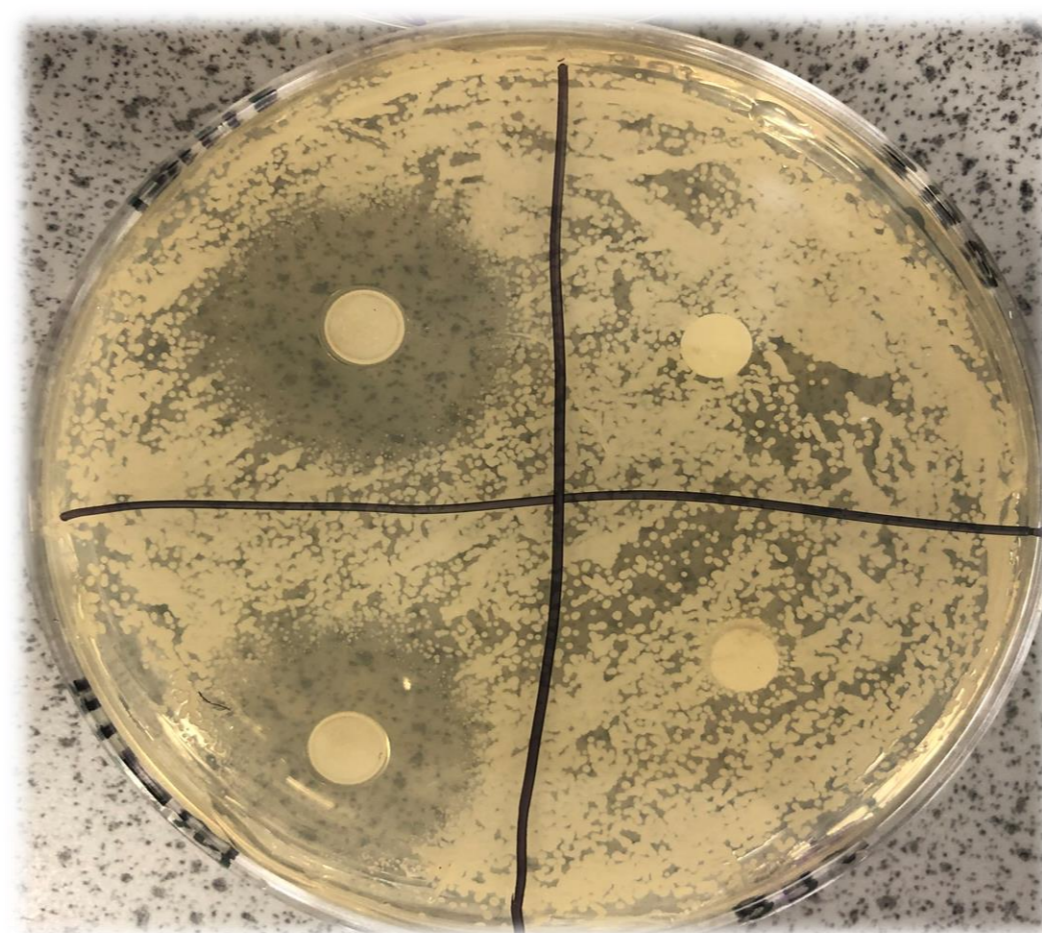


Figure 3 Disc diffusion assay

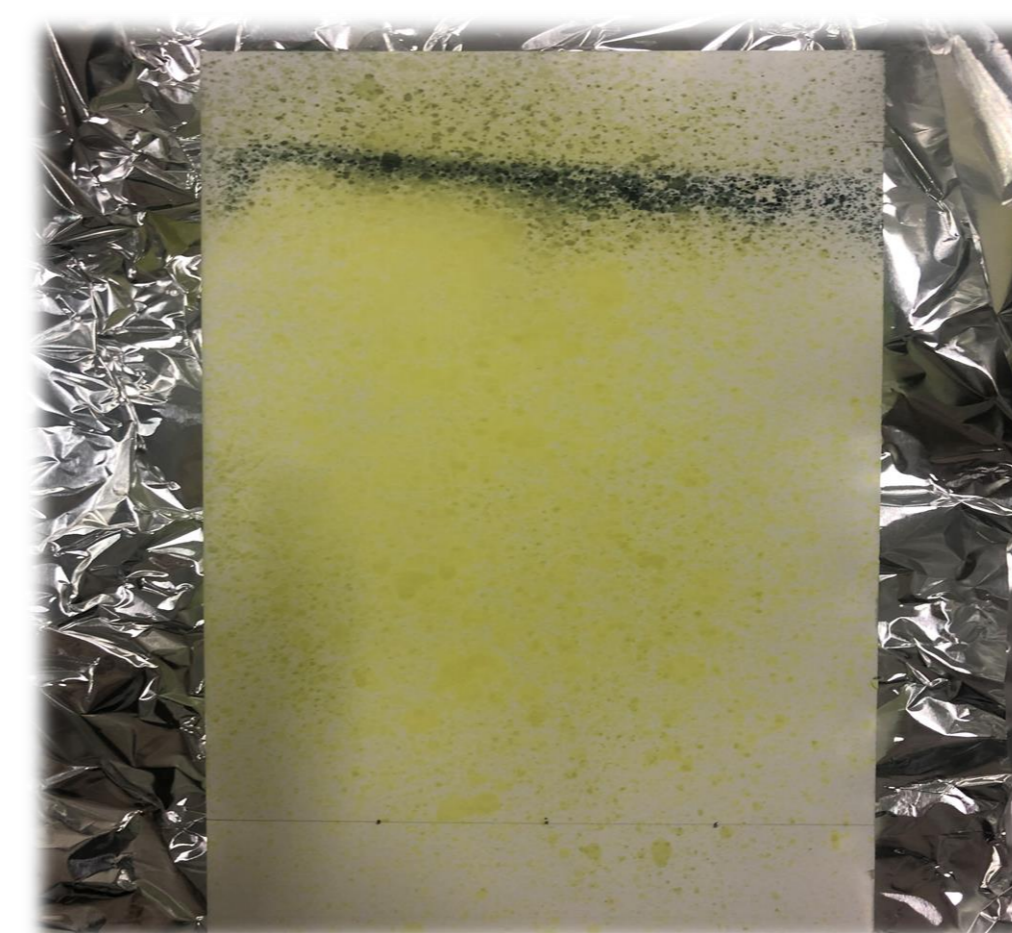


Figure 4 Thin layer chromatography



Figure 5 Acid value assay performed in the TUS research hub



Figure 6 Bomb calorimeter located in TUS midlands campus

CONCLUSION

Although this research group is armed with a promising antifungal market contender, gaps in the knowledge still exist. The modification process of the coconut oil must be proved/validated, by identifying the components that allow the oil to triumph over the *T. rubrum* infection. The hope is to fill these knowledge gaps by employing effective methods and then address any remaining barriers to commercialisation.