Investigating the potential of Manuka oil as a novel topical scabicide

Nirupama Nammunige[1], Kylie Agnew-Francis[2], Deepani D. Fernando[1], Sara Taylor[1], Hieng Lu[1], Gangi Samarawickrama[1], Craig Williams[2], Robin B. Gasser[3] and Katja Fischer[1]

[1]Cellular and Molecular Biology Department, Infectious Diseases Program, QIMR Berghofer Medical Research Institute, Brisbane, Australia
[2] School of Chemistry and Molecular Biosciences, The University of Queensland, Queensland, Australia
[3]Faculty of Veterinary Science and Agricultural Sciences, The University of Melbourne, Melbourne, Australia

Background

- Scabies is common in overcrowded and socioeconomically disadvantaged settings, both in the Australian Aboriginal population and worldwide, with 300 million cases reported annually. Scabies is caused by infestation with the parasitic mite *Sarcoptes scabiei* (Figure 1).
- Two main disease manifestations are recognised. Ordinary scabies is commonly presented, generally entailing a low parasite burden of <20 mites/patient, causing lesions and a generalized intense allergic rash, resulting from inflammatory and hypersensitivity reactions to the mites and their products. Crusted scabies is a relatively rare, life-threatening and poorly understood disease manifestation, frequently associated with an immunosuppression. Importantly, scabies is often linked to secondary infections with opportunistic bacteria.
- There is no vaccine and there are only a few broad-spectrum anti-parasitic drugs to combat this highly contagious disease like topical Permethrin, oral Ivermectin, benzyl benzoate, crotaminton etc. Available drugs kill the motile parasite stages (larvae, nymphs and adults) mainly by interfering with the mite's nervous system and muscle function. Scabies is not often cured by these drugs, because of the eggs are not susceptible to there treatment and drugs have short skin half-lifes.
- Mite resistance against drugs is emerging, which also emphasizes the urgency of finding novel scabicides to transform scabies treatment and management at the individual, household and community levels.
- The Manuka tree (*Leptospermums coparium*) is indigenous to New Zealand, and its essential oil has traditionally been used by the Maori people to treat wounds. It has been reported to have antimicrobial and acaricidal properties against a range of pest mites, however, its efficacy as an ovicide and miticide on scabies has not been reported previously, and the active chemical(s) have not been identified. We propose that its natural products, and/or derivatives will provide next generation candidates for a topical scabies treatment.

OBJECTIVES:

(1) To identify the active natural chemical agent(s) of Manuka oil with ovicidal and miticidal activity against *Sarcoptes scabiei*

(1.1)To determine the LC50 value of active chemical agents of Manuka oil on mite killing and egg hatching

(1.2) To verify possible synergistic effect of active chemical agents in Manuka oil

(1.3) To determine the LT50 (optimum exposure time) of the best active compound of Manuka oil on mite killing and egg hatching

(2) To elucidate the mode(s) of action of Manuka oil/ best active compounds

(2.1) To analyse the embryonic development (morphological changes) of *Sarcoptes scabiei* eggs exposed to Manuka oil/active chemical compounds

(3) Conduct preclinical trials to assess the efficacy of selected lead compounds

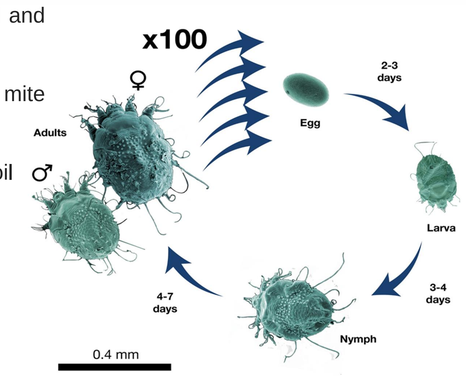
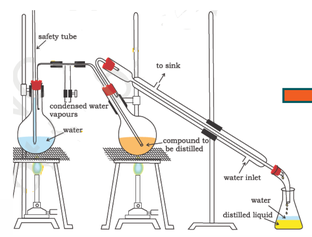


Figure 1: *Sarcoptes scabiei* life cycle (Bernigaud *et.al* 2019)

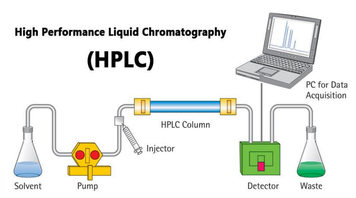
Methodology

AIM 1: Activity-guided isolation of scabies miticides and ovicides from neat Manuka oil and targeted *in vitro* screening of related active agent(s)

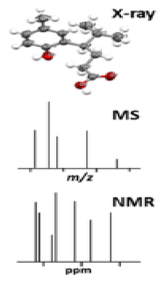
Neat Manuka Oil (Phytomed Medicinal Herbs Ltd)



Steam/Vaccum Distillation



High Performance Liquid Chromatography



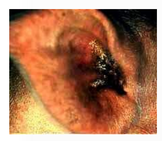
Structural Elucidation

***In vitro* bio assay**

AIM 2: Elucidate pathways and/or biological targets affected by Manuka oil/ active agents

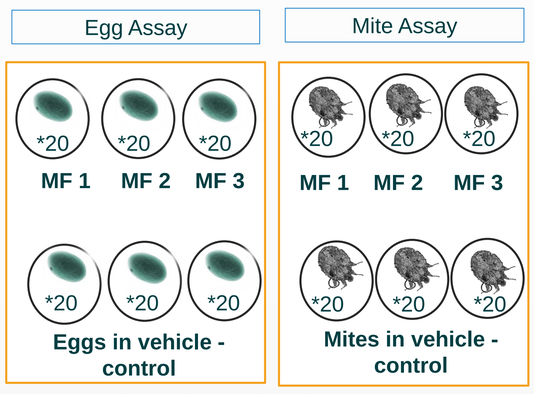
Proteo-transcriptomic comparative analysis of mites *vs* eggs

Eggs and mites for the bio assays – *Sarcoptes scabiei* var *suis*



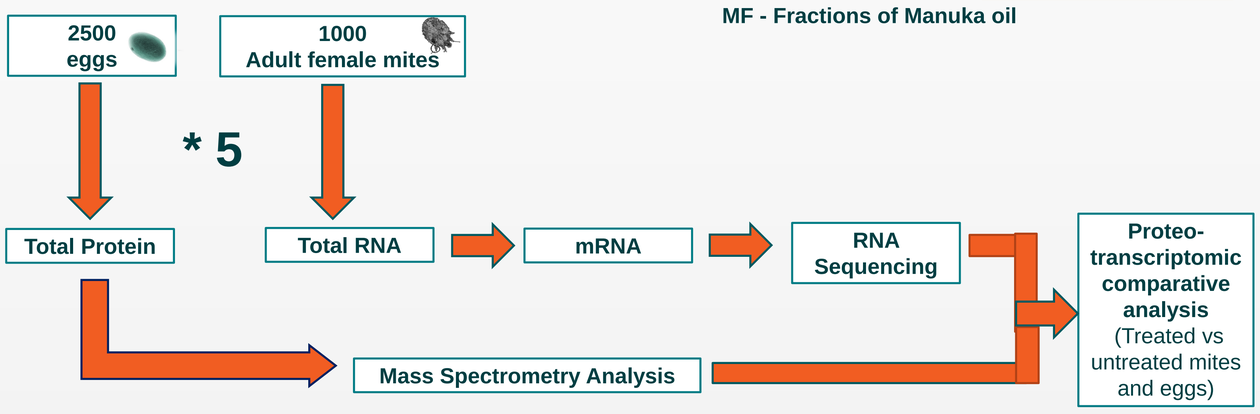
Pig Ear Scraping → Crusts → Eggs / Mites

***In vitro* bio assay for determining treatment conditions of Manuka oil**

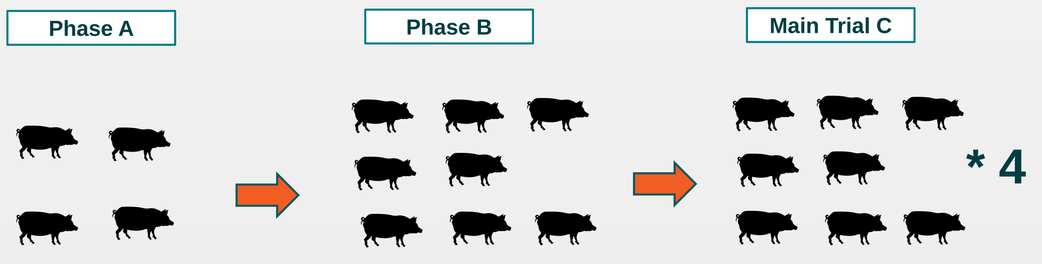


MF - Fractions of Manuka oil

Transcriptomic and Proteomics



AIM 3: Pre-clinical trials to assess the efficacy of prioritized compounds

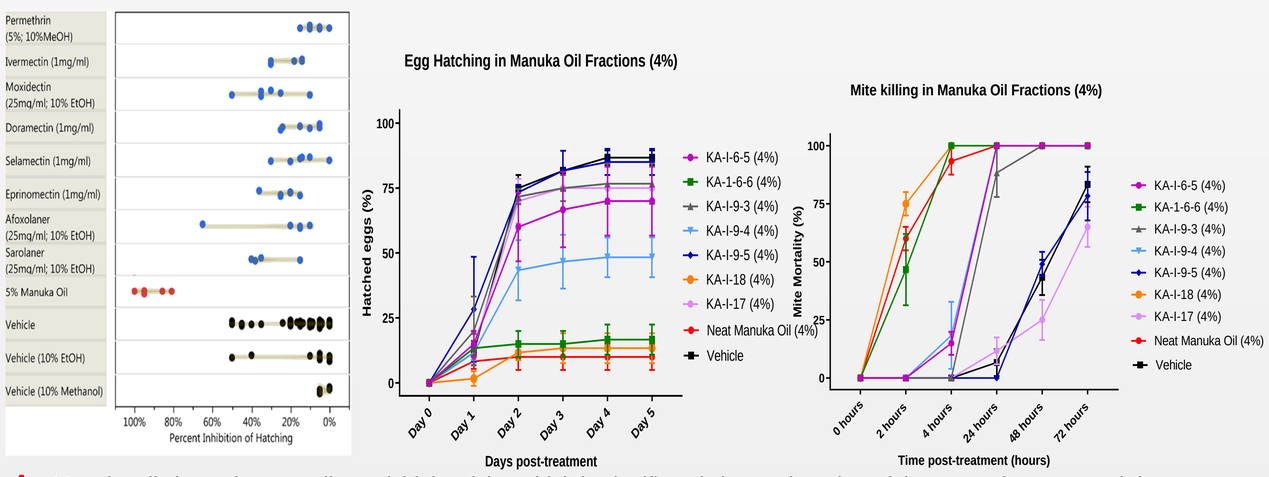


4 pigs (2 treated, 2 untreated) to assess tolerability of the test formulation, to obtain an early indication of the *in vivo* potential for this drug candidate to control scabies and to justify the large numbers of animals sacrificed in phase B and main trial C.

8 pigs with advanced mite infestation are required to provide sufficient parasite material for main drug trial C.

32 pigs will be infected with a standard dose of 1000 mites and randomly allocated into 4 groups of 8 pigs each. Mite counts will be examined on days 0, 7, 14, 28, and 42 in samples from an area not previously scraped. Skin samples are examined under a stereomicroscope and live parasites are counted twice. Skin condition, pruritus and weight gain will be monitored weekly from infection day to end of trial.

Preliminary Data



A - Manuka oil showed outstanding ovicidal activity, which is significantly better than that of the currently most used drugs.

B - The above graphs show the ovicidal and miticidal activity of various Manuka Oil fractions generated from neat Manuka oil. Both graphs show the activity of these fractions at 4% in comparison with a control and the neat Manuka oil, as can be seen there are two fractions that show outstanding scabicidal activity (KA-I-18 , KA-I-6-6) with approximately 15% egg hatching and 100% mite mortality in four hours.

Expected Outcomes

Pre-clinical *in vivo* data comparing the efficacy of a novel scabicide that kills mites and eggs to currently recommended treatments against scabies. Fundamental knowledge about the effect of this drug on candidate target mite proteins.

References

Bernigaud, C., Samarawickrama, G.R., Jones, M.K., Gasser, R.B. and Fischer, K., 2019. The challenge of developing a single-dose treatment for scabies. *Trends in parasitology*, *35*(11), pp.931-943.

Bernigaud, C., Fang, F., Fischer, K., Aho, L.S., Dreau, D., and Botterel, F., 2016. Preclinical study of single-dose moxidectin, a new oral treatment for scabies: efficacy, safety, and pharmacokinetics compared to two-dose ivermectin in a porcine model. *PLoS neglected tropical diseases*, *10*(10), p.e0005030.

Fernando, D.D., Marr, E.J., Zakrzewski, M., Reynolds, S.L., Burgess, S.T. and Fischer, K., 2017. Gene silencing by RNA interference in Sarcoptes scabiei: a molecular tool to identify novel therapeutic targets. *Parasites & vectors*, *10*(1), p.289

Acknowledgement

Phil Rasmussen, Phytomed medicinal Herbs Ltd & Kiwiherb, Auckland, New Zealand

Prof. Katherine Andrews, Griffith University

Prof. Peter Parsons, QIMR Berghofer MRI

Dr. Simone Reynolds, Griffith University