Kawakawa extracts demonstrate anti inflammatory activity

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Introduction

In traditional Māori medicine (rongoā) Kawakawa *(Macropiper excelsum)* is used to treat a wide variety of ailments. It is considered to be one of the most potent medicinal herbs[1].

There has been very little previous research into Kawakawa. Previous screening studies have indicated that Kawakawa had little anti-bacterial and anti-viral activity[1,2]. Evidence suggests that the extraction methodologies used in these previous studies were not the most suitable, as both used organic solvents[1,2] whereas traditional Māori preparation used water[3]. This could account for the disparity between what was observed in rongoā and the previous scientific studies. There has been no previous research into Kawakawa's anti-inflammatory properties.

This study sought to investigate the disparity between the anecdotal evidence in rongoā and the scientific evidence available. It was hypothesised that Kawakawa will have anti-inflammatory activity, providing scientific support for the its use in rongoā.

Conclusions

Kawakawa has anti-inflammatory activity at specific concentrations of aqueous extract. The results show that:

- **Anti-inflammatory activity was only observed in the aqueous extract.**
- **The aqueous extract caused a dose-dependent decrease in nitric oxide, TNF-α and Il-6 production.**
- **Nitirc oxide production was supressed at concentrations of 1000 µg/mL and 500 µg/mL**
- **The inhibition of Il-6 production was maximal at extract concentrations of 1000 µg/mL and 500 µg/mL.**
- **This inhibition of TNF-α production was maximal at extract concentrations of 250 µg/mL and 125 µg/mL.**

Many of the traditional uses of Kawakawa could be linked directly to inflammation (such as toothache, irritation, serious bruises)[3]. The anti-inflammatory actions of Kawakawa could mask the symptoms of ailments not directly associated with inflammation (such as viral infections).

This study fills a niche in the literature as there been no previous research into the anti-inflammatory properties of Kawakawa, nor has any other research provided a scientific basis that supports the actions of Kawakawa in rongoā. Therefore, the uses of Kawakawa identified by Māori in rongoā are supported by the anti-inflammatory activity observed in this study.

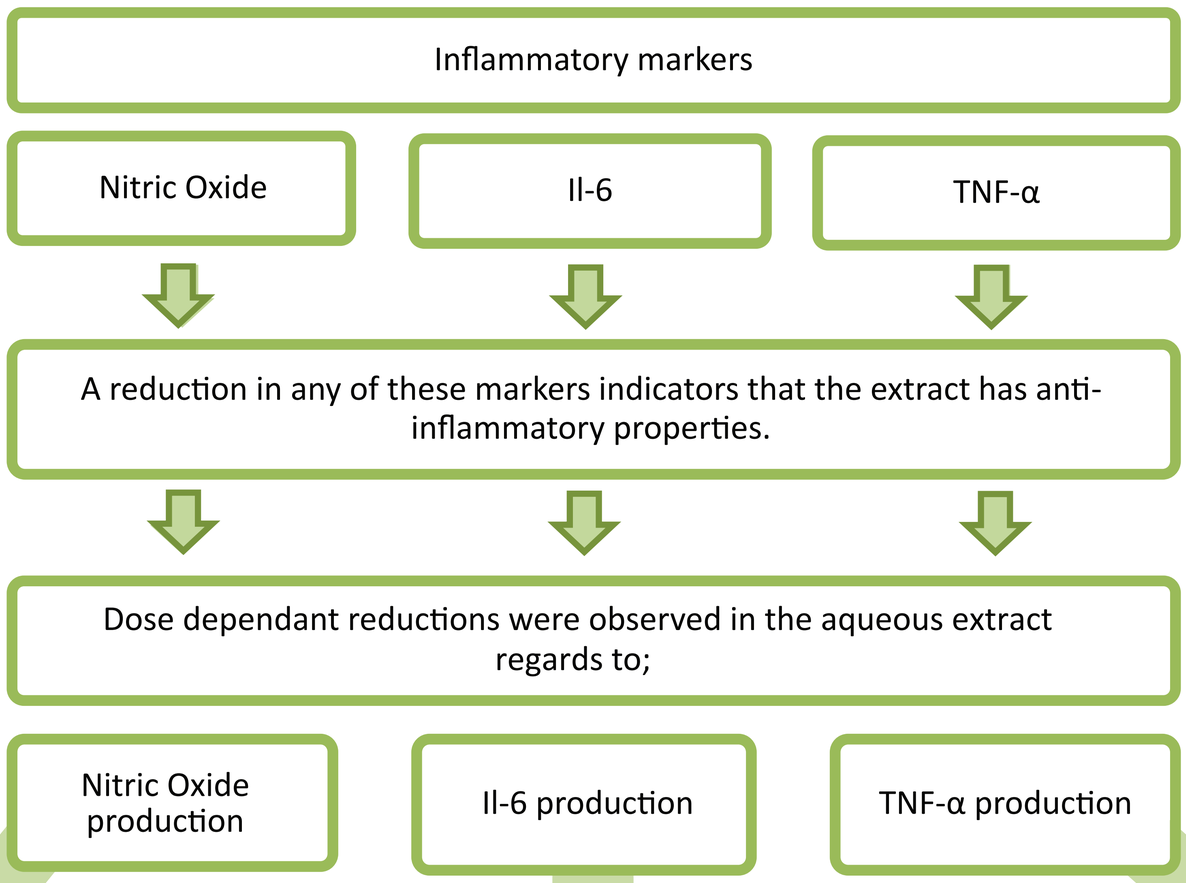
Discussion

With regards to the extractions:

- Only the aqueous extract demonstrated anti-inflammatory activity.
- This methodology best represented traditional Māori methods.[3].
- The extractions suggest the compounds responsible for the bioactivity are thermostable and polar.
- The evidence suggests that the extract contained a variety of active compounds as it acted as both a pro and anti-inflammatory agent in some assays.
- This suggests that the results arise from interactions between pro and anti-inflammatory compounds.

Acute inflammation is associated with the production of reactive oxygen species such as nitric oxide, which is considered to be a key inflammatory marker:

- There were reductions in the nitric oxide production at the two highest concentrations of the aqueous extract (figure 1).
- This suggests that the activity is dose dependant.



The inflammatory cytokine TNF-α is a second key inflammatory marker:

- The TNF-α results (figure 2) closely replicated those of nitric oxide assay meaning the chlorofrom-methanol-water extract showed no activity.
- The aqueous infusion extract caused statistically significant ($P<0.05$) reductions in TNF-α production at concentrations of 500 µg/mL and 1000 µg/mL.
- The aqueous infusion extract at a concentration of 15.6 µg/mL caused a statistically significant ($P<0.05$) increase in TNF-α production. This was unexpected.
- This suggests that the activity is does dependant.

The inflammatory cytokine Il-6 is another key inflammatory marker:

- The aqueous extract caused a slight reduction in Il-6 production at concentrations of 250 µg/mL and 125 µg/mL.
- The TNF-α and Il-6 assays behaved differently. This suggests that the extract affects each of the intracellular mechanisms responsible for the production for each of the cytokines differently.

Results

A positive result is a reduction in the value compared to the control. A reduction in nitric oxide, TNF-α and Il-6 production would be indicative of anti-inflammatory activity. This was observed in the aqueous extract.
* Indicates statistical relevance ($P<0.05$). Statistical significance calculated using a T-test. 0 µg/mL of Kawakawa extract was the control in all samples. Error bars show the standard error of the mean.

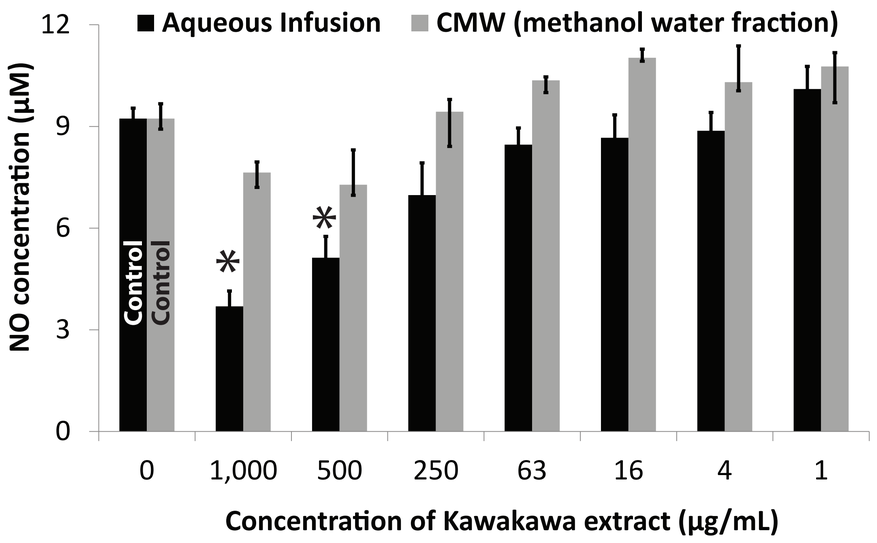


Figure 1: Nitric oxide produced in a variety of Kawakawa extracts: Reductions in the aqueous extract at the concentrations of 1000 µg/mL, and 500 µg/mL.

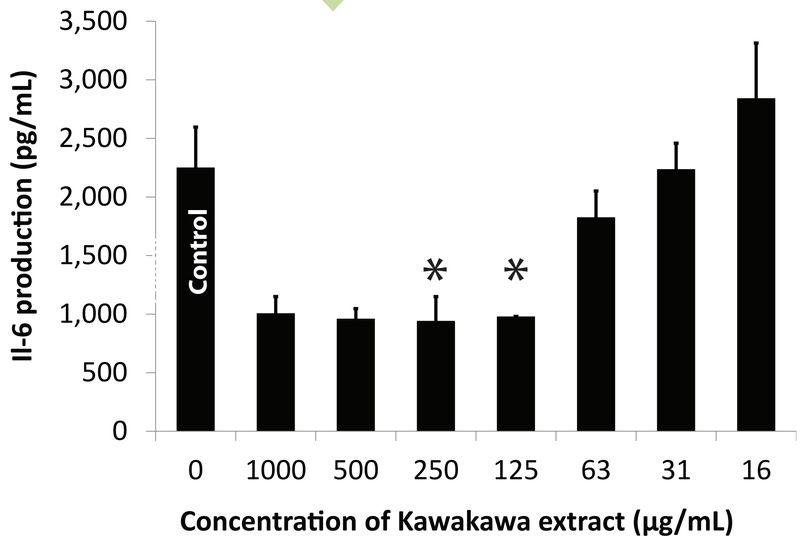


Figure 2: Il-6 production in cells exposed to the aqueous infusion extract: Reductions in the aqueous extract at the concentrations of 250 µg/mL and 125 µg/mL.

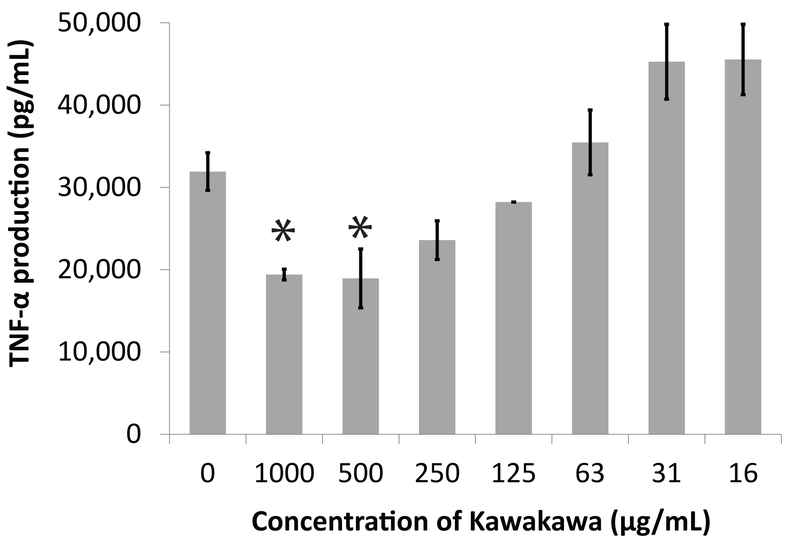
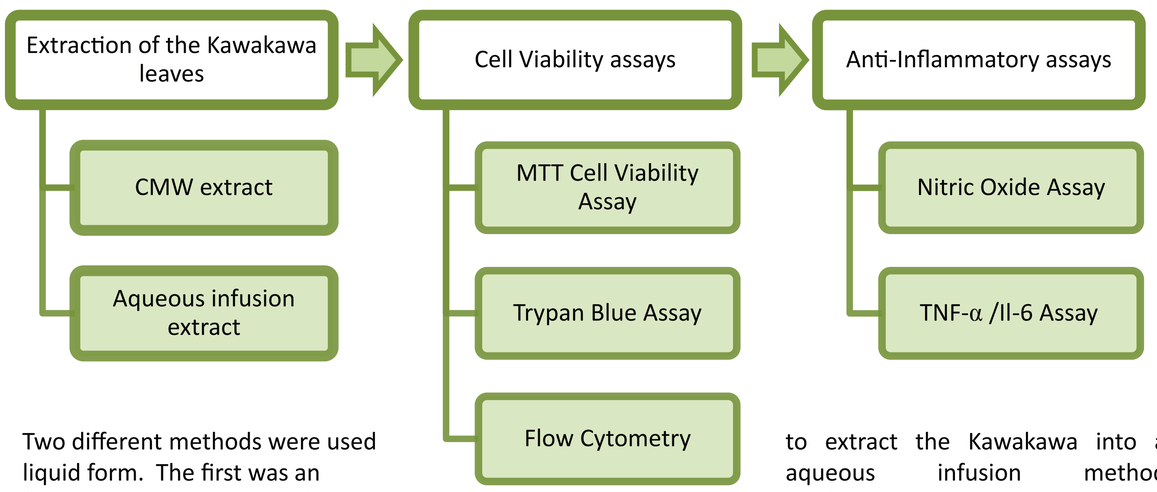


Figure 3: TNF-α production in cells exposed to the aqueous infusion extract: Reductions in the aqueous extract at the concentrations of 1000 µg/mL and 500 µg/mL.

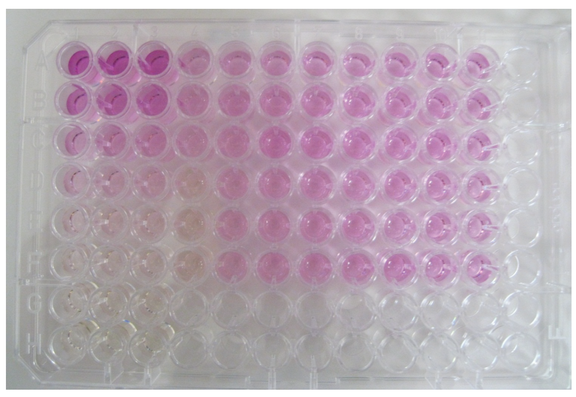
Methodology



Two different methods were used to extract the Kawakawa into a liquid form. The first was an aqueous infusion method (analogous to making a tea) and similar to methods used in rongoā. In the second, leaves were soaked in chloroform-methanol-water (1:2:1 volume to volume).

The extract was tested for cytotoxicity in cell viability assays and then the non-toxic concentrations were used in the anti-inflammatory assays.

Kawakawa leaves like those collected and extracted for use in this study. Photo by Chris Ryan.



A 96 well plate used in the nitric oxide assay. The darker the colour the greater the nitric oxide concentration. Photo by Chris Ryan.

References & Acknowledgments

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References:

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2. Calder, V. L., Cole, A. L., & Walker, J. R. (1986). Antibiotic compounds from New Zealand plants. III: A survey of some New Zealand plants for antibiotic substances. *Journal of the Royal Society of New Zealand, 16*(2), 169-181.
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