## 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<sup>1</sup>.

There has been very little previous research into Kawakawa. Previous screening studies have indicated that Kawakawa had little anti-bacterial and anti-viral activity<sup>1,2</sup>. Evidence suggests that the extraction methodologies used in these previous studies were not the most suitable, as both used organic solvents<sup>1,2</sup> whereas traditional Māori preparation used water<sup>3</sup>. 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- $\alpha$  and II-6 production.
- Nitirc oxide production was supressed at concentrations of 1000  $\mu$ g/mL and 500  $\mu$ g/mL
- The inhibition of II-6 production was maximal at extract concentrations of 1000  $\mu$ g/mL and 500  $\mu$ g/mL.
- This inhibition of TNF- $\alpha$  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)<sup>3</sup>. The antiinflammatory 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.

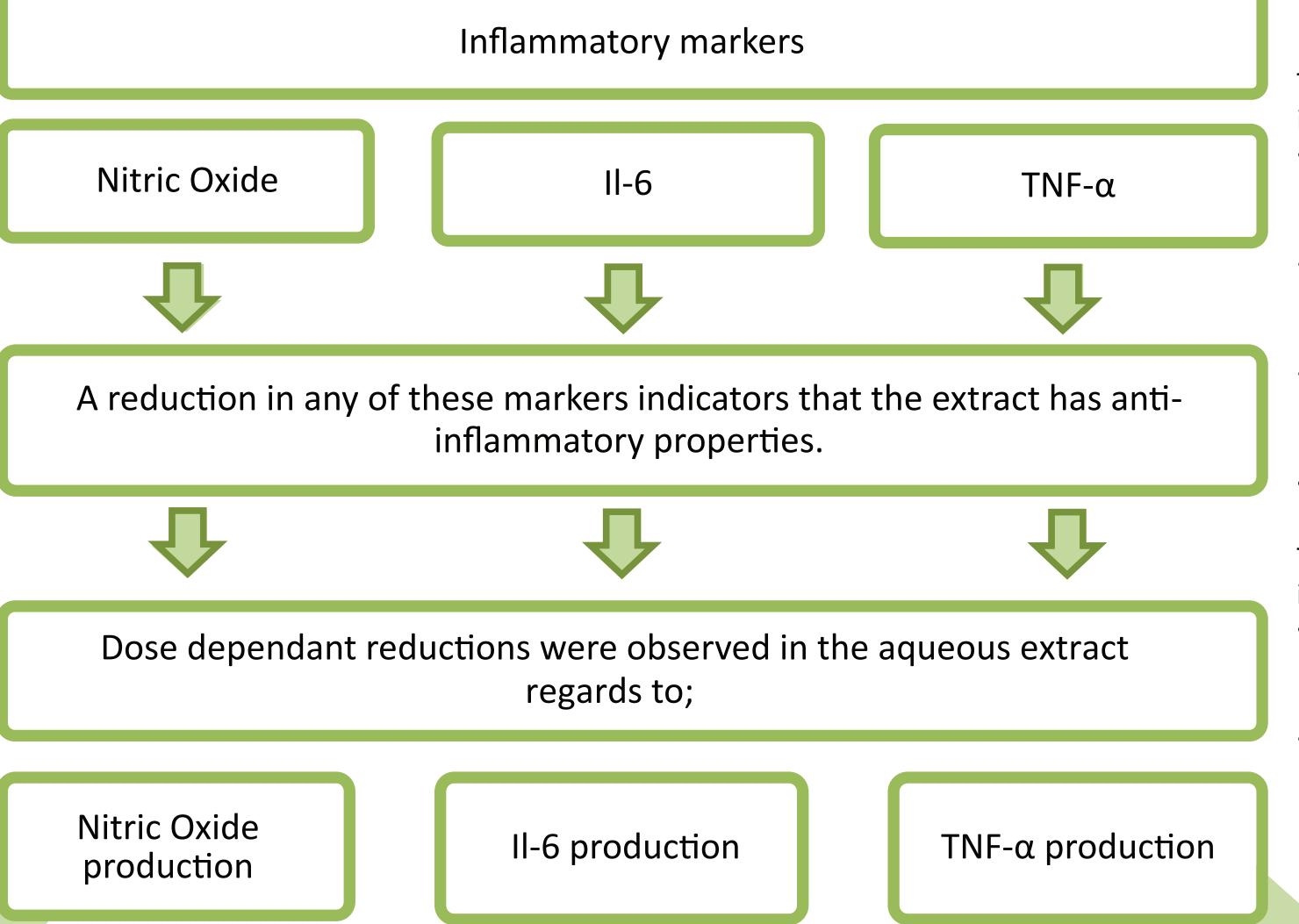
# Discussion

With regards to the extractions:

- Only the aqueous extract demonstrated antiinflammatory activity.
- This methodology best represented traditional Māori methods.<sup>3</sup>.
- The extractions the suggest 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 dependent.



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Kawakawa

extracts

demonstrate

anti inflammatory

activity

The inflammatory cytokine TNF- $\alpha$  is a second key inflammatory marker:

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

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

- The aqueous extract caused a slight reduction in II-6 production at concentrations of 250  $\mu$ g/mL and 125 μg/mL.
- The TNF- $\alpha$  and II-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 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.

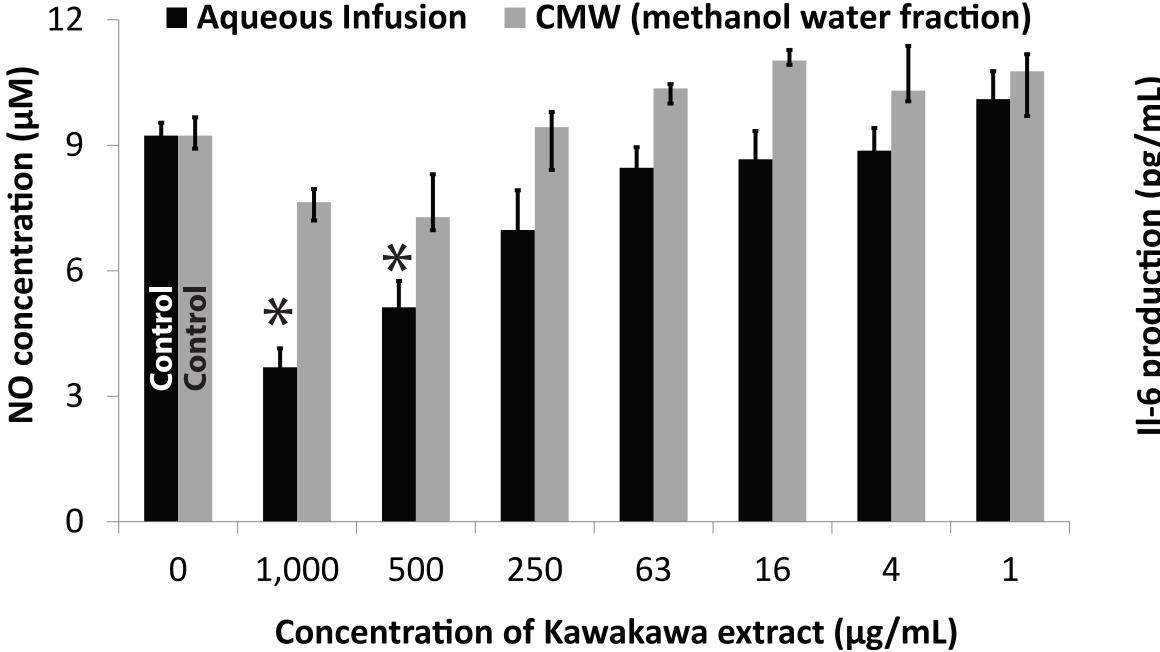
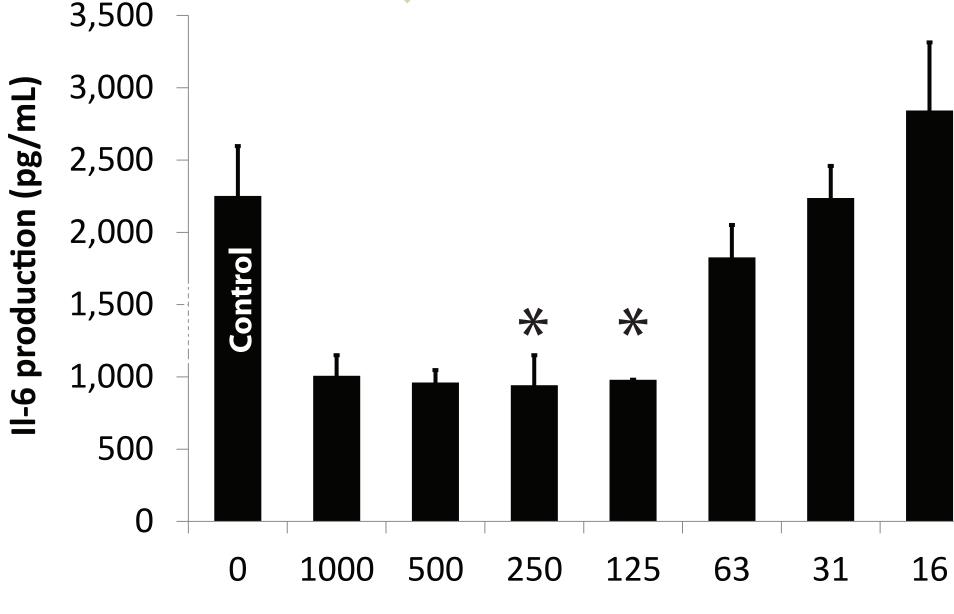


Figure 1: Nitric oxide produced in a variety of Kawakawa extracts: Reductions in the aqueous extract at the concentrations of 1000  $\mu$ g/mL, and 500  $\mu$ g/mL.



Concentration of Kawakawa extract (µg/mL)

**Figure 2: II-6 production in cells exposed to the aqueous** infusion extract: Reductions in the aqueous extract at the concentrations of 250  $\mu$ g/mL and 125  $\mu$ g/mL.

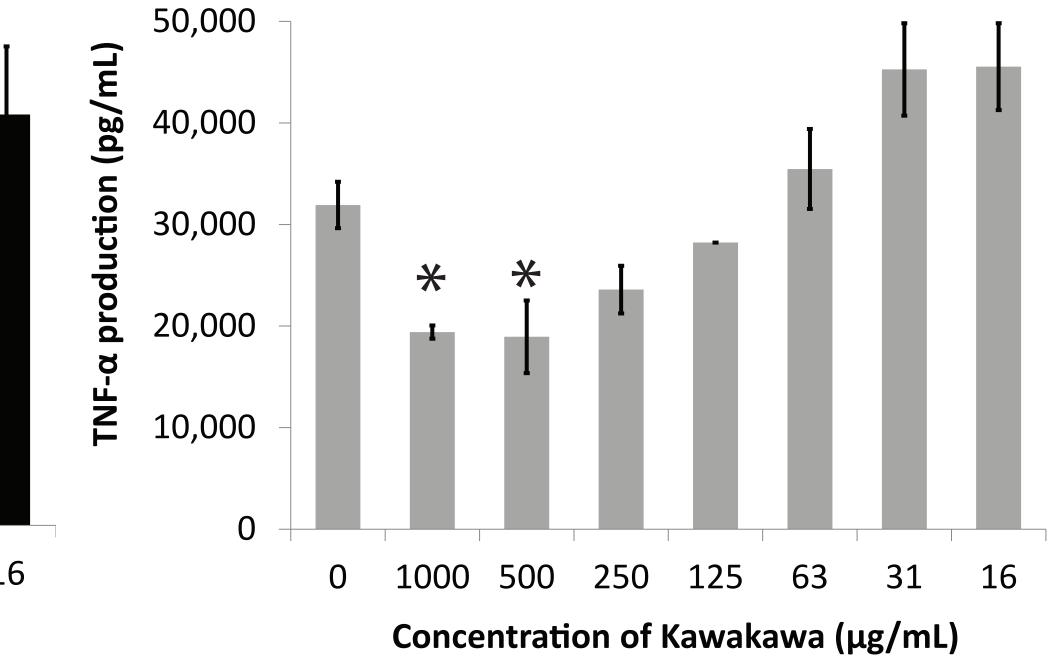
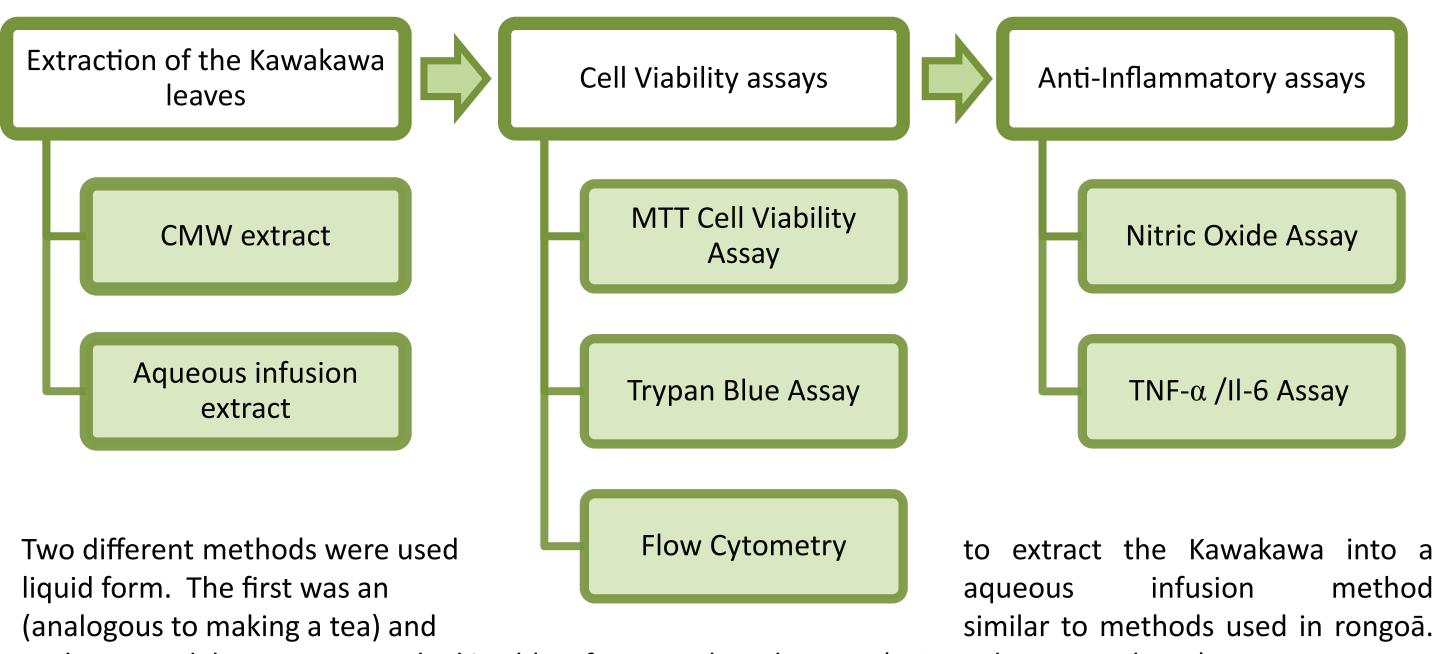


Figure 3: TNF-α production in cells exposed to the aqueous infusion extract: Reductions in the aqueous extract at the concentrations of 1000  $\mu$ g/mL and 500  $\mu$ g/mL.

#### Methodology





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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