# A COMPARISON OF BELOWGROUND AND ABOVEGROUND ANTIMICROBIAL ACTIVITY IN *HIBISCUS TILIACEUS*, *HIBISCUS ROSA-SINENSIS* AND *THESPESIA POPULNEA* AND ITS CORRELATION WITH ROOT DECOMPOSITION AND SOIL NUTRIENT CONTENT IN MOOREA, FRENCH POLYNESIA

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*Abstract.* As belowground interactions of plants are often neglected, this study aimed to analyze and compare the belowground and aboveground magnitude of anti-microbial activity of three medicinal plants of the Malvaceae family. *Hibiscus tiliaceus, Hibiscus rosa-sinensis* and *Thespesia populnea* were screened for antimicrobial activity using an antimicrobial bioassay to measure baking yeast inhibition. Roots of all species were set to decompose at one-week intervals for three weeks to correlate belowground antimicrobial activity with root decomposition. Soil tests were performed at different sites to correlate belowground antimicrobial activity with soil nutrient content. This study showed that belowground and aboveground antimicrobial activity did not differ in each species, nor were there differences among species. There was also no significant correlation between belowground antimicrobial activity and root decomposition or soil nutrient content presumably because of the lack of soil diversity in Moorea, French Polynesia. Future studies should consider testing the antimicrobial activity of these medicinal plants on microorganisms that more commonly affect humans as opposed to baking yeast.

*Key words: medicinal plants*; Hibiscus tiliaceus, Hibiscus rosa-sinensis, Thespesia populnea; *root decomposition; soil nutrient content; South Pacific*

## INTRODUCTION

In many developing countries, traditional medicine has been used as much as modern pharmaceuticals due to its affordability and accessibility (Bussmann et. al 2009). People have often turned to plants for treatment because they are natural and thought to be free of side-effects (Bussmann et. al 2009). The majority of the people of the South Pacific believe that minor injuries and familiar illnesses are best treated utilizing traditional medicine as opposed to Western medicine (Anonymous 1998). Whistler (1992) described traditional Tahitian medicine as the use of herbal remedies to address ailments, or the performance of certain activities to address supernatural influences and external forces.

The Antimicrobial activity in plants has been linked to secondary compounds such as alkaloids, coumarins, isoflavonoids, polyacetylenes, quinones, tannins and terpenes (Reichling 2010). Plants use these secondary compounds for defense strategies (Reichling 2010), enabling tolerance, indirect resistance by enhancing the action of enemies of herbivores, or chemical and mechanical traits that reduce herbivory (Trotter 2005). Secondary compounds used for defense mechanisms are also dependent on resource availability (Coley et al. 1985). Coley et al. (1985), found that fast growing plants adapted to resource-rich habitats suffered less from herbivore damage and had lower amounts of defensive chemicals than slow-growing species in resource-poor habitats. Appel et al. (2006) found the same relationship in plants of the Amazonian forest, suggesting a universal trade-off that plants in nutrient poor soils follow the growth-defense trade-off of allocating more resources, such as secondary compounds, to defense (Appel et al. 2006). Some of these plant-derived secondary compounds are the same biologically active compounds found in pharmaceuticals (Balandrin et. al 1985).

In previous studies, plant secondary compounds were linked to the rate of decomposition (Paul 2006). Decomposition occurs in a two-phase process with the first phase characterized by the rapid loss or leaching of easily decomposed components including secondary compounds used in defense mechanisms (Cates et al. 1988). In

many systems, secondary compounds such as tannins, have been used to measure decomposition rates (Cates et al. 1988). However, little is known about the allocation of secondary compounds in the roots of plants (Cates et al. 1988). Previous studies by Blossey and Notzold (1995) found that there were less secondary compounds used for defense in non-indigenous plants as explained by the evolution of increased competitive ability hypothesis. This hypothesis predicted that in the absence of herbivores, selection favored genotypes with improved competitive abilities and allocated fewer resources to herbivore defense (Blossey and Notzold 1995).

Agrawal and Rassmann (2008) found that aboveground and belowground parts expressed defensive phytochemical compounds in equal concentrations. Studies by Priya and Sejal (2012) showed the opposite effect where the roots of *H. rosa-sinensis* had the greatest growth inhibition on various microorganisms. Furthermore, Bowers et al. (1998, 1999, 2002) showed that phytoecdysteroids, a pharmacological chemical that plants synthesize for defense (Cheng et al. 2009), increased only in roots and not shoots after herbivore damage in spinach. Because of these conflicting studies, the antimicrobial activity of plants of the Malvaceae family: *Hibiscus tiliaceus*, a native tree of Moorea, *Hibiscus rosa-sinensis*, an introduced shrub species, and *Thespesia populnea*, a native tree, used in traditional medicine throughout Moorea, French Polynesia (Anonymous 1998), were used to compare belowground antimicrobial activity to the aboveground antimicrobial activity of secondary compounds.

The overall goal of this study was to compare the belowground antimicrobial activity to the aboveground antimicrobial activity of *Hibiscus rosa-sinensis, Hibiscus tiliaceus* and *Thespesia populnea.* This study also focused on correlating antimicrobial activity to both root decomposition and soil nutrient content. It was hypothesized that (1) the magnitude of antimicrobial activity would be greater belowground than aboveground for all study species based on previous studies by Priya and Sejal (2012) (2) the native *H. tiliaceus* and *T. populnea* would have greater antimicrobial activity than the introduced *H.rosa-sinensis,* based on the evolution of increased competitive ability hypothesis (3) low soil nutrient levels would result in increased antimicrobial activity, based on the growth-defense trade-off of allocating secondary compounds to defense (Appel et al. 2006) (4) greater belowground antimicrobial activity would correlate with a slower decomposition rate in roots since secondary compounds are related to the rate of decomposition (Paul 2006).

## METHODS

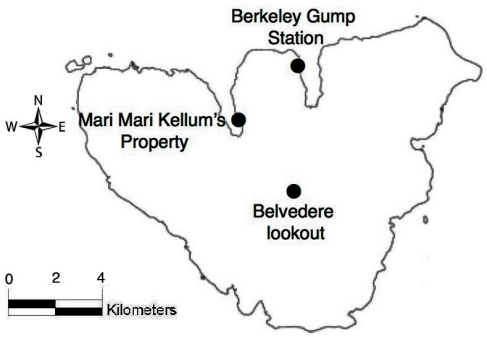


FIG 1. Site locations on Moorea, French Polynesia

### *Study site*

*Hibiscus tiliaceus*, *Hibiscus rosa-sinensis* and *Thespesia populnea* were collected along the coast of the UC Berkeley Gump Station (17°29′28.58″S, 149°48′35.43″W) and Mari Mari Kellum's property (17°30′51.34″S, 149°50′53.42″W). As *T. populnea* was absent at the Belvedere lookout (17°32′25.79″S, 149°49′36.08″W), a site with a higher elevation (300m), only *Hibiscus tiliaceus* and *Hibiscus rosa-sinensis* were collected from this site. A complete list of medicinal uses for each plant part has been summarized in appendix A. All plant species collected were deposited in the University of California Berkeley Herbarium.

### *Belowground and aboveground antimicrobial assays*

The following methodology for extract preparation and the antimicrobial assay was adopted from Cox (2008) with suitable modifications. Five grams of fresh root matter were collected, weighed, rinsed with water, cleaned with a Kim Wipe™ that ha been dipped in ethanol and sliced into thin pieces. To make ethanol and water extracts, the 30mL of 90% ethanol or 30mL of deionized water were added to a blender containing the plant matter and mixed to obtain a homogenous mixture. The plant extracts were then transferred to sterile vials using a sterile

pipette, refrigerated at 7°C and shaken daily for three days before they were used in the antimicrobial assay (described below). Both water and ethanol extracts were used since studies by Cox (2008) showed that water extracts yielded a higher overall mean inhibition than ethanol extracts. The same procedure was used to test the antimicrobial properties of the aboveground parts (leaves, flowers and fruits) in water extracts. Parts of plants collected appeared healthy, free of disease and herbivory. Five replicates were used per study site.

A culture of baking yeast (*Saccharomyces cerevisiae*), was used for the antimicrobial assay due to its accessibility. Sugar enriched agar medium was made by mixing 14g of gum agar powder with 100g of white granulated sugar and 1L of deionized water in a large Erlenmeyer flask. The sugar enriched agar was then autoclaved at 121°C for 15 minutes at 0 PSI. The agar medium was then distributed into 100x15mm Petri dishes and allowed to solidify overnight. The Petri dishes were then inverted to reduce condensation and stored at room temperature until use.

To set up the antimicrobial assay, 3g of baking yeast were mixed with 50mL of deionized water in a sterile container. Sterile cotton swabs were used to spread the yeast evenly onto each Petri dish. Each Petri dish containing yeast was then covered with Parafilm and incubated at 30°C for 24 hours to attain a uniform spread over the agar medium before the extracts were applied.

Filter paper discs with a diameter of 0.6cm were made using a hole puncher and then sterilized in the autoclave. Each sterile disc was then dipped three times in water or ethanol plant extracts. A total of ten discs were evenly placed in the surface of each yeast inoculated Petri dish. Deionized water and ethanol were used as negative controls and the commercial anti-fungal, 1% econazole nitrate, was used as the positive control. After the plates were inoculated with the extracts, the plates were re-sealed with Parafilm and incubated for three days at 30°C. Yeast inhibition was characterized as a clear zone of inhibition surrounding the extract soaked filter paper discs. Yeast inhibition was then measured qualitatively by assigning a rating of "0" for no inhibition, "1" for low inhibition, "2" for medium inhibition, and a "3" for high inhibition. The inhibition ratings were then averaged for each Petri dish.

*Soil nutrient content*

One cup of soil was collected from all study sites, taken back to the lab, left to dry overnight and passed through a sieve to remove any stones or sticks. A LaMotte NPK Soil Test Kit was used to measure the nitrogen, phosphorus and potassium levels. Three soil tests were done per site.

*Decomposition of Belowground Biomass*

Fine roots of *H. tiliaceus*, *H. rosa-sinensis*, and *T. populnea* were excavated and collected from the three study sites, with the exception of the Belvedere lookout, where *T. populnea* is absent. The roots of each species were rinsed with water to remove dirt and then dried for three days in a homemade oven made from four 60-watt lamps in a large wooden chamber. Then, 1g of root material for each species per site were placed in individual 10cm by 15cm mesh bags. Since root decomposition was observed over the course of three weeks then three sets of roots (each containing 1g) for week one, two and three, were made to deploy in individual 10cm by 15cm mesh bags for each species per site. All mesh bags containing root material were labeled with the species name and site they were collected from and finally buried at a 45° angle 10cm belowground. To eliminate the possibility of abiotic factors affecting root decomposition, all mesh bags were buried at the Berkeley Gump Station for one, two and three weeks. At the appropriate time, the mesh bags were recovered, opened, the root material was rinsed with water, dried at 30°C for three days and weighed again to determine the root mass loss (root decomposition) for each plant species per site.

*Statistical Analyses*

All statistical analysis of data was done using R (R Development Core Team, version 0.97.551, 2013). A one-way ANOVA and a Tukey test were used to test for differences between different variables such as solvent (water and ethanol), plants (*H. rosa-sinensis, H. tiliaceus* and *T .populnea*), parts (roots, leaves, flowers and fruits) and study sites (Berkeley Gump station, Mari Mari Kellum's property and the Belvedere lookout) on yeast inhibition.

A one-way ANOVA and a Tukey test was also used to analyze the effects of two variables; plants (*H.rosa-sinensis, H.tiliaceus*

and *T. populnea*) and weeks (one, two or three) on root decomposition.

A Shapiro Wilk test was done to test for normality between decomposition rate and belowground antimicrobial activity. Correlation tests using the Pearson method were used to analyze the relationship between decomposition rate and belowground antimicrobial activity.

A fligner test was used to test for homogeneity of variances in comparing average root mass loss week and species. As it was not significant, a two-way ANOVA was used to test for differences between week and species.

## RESULTS

### *Belowground and aboveground antimicrobial assays*

Yeast inhibition was not significantly affected by the solvent type across all plant parts (GLM (quasipoisson), $F_{1,101} = 0$, $p = 1$). Ethanol had a mean inhibition of 0.7 (± SD 0.78) while water had a mean inhibition of 0.58 (± SD 0.79). Yeast inhibition was not significantly affected by the study site (ANOVA, $F_{2,77} = 1.08$, $p = 0.34$). The average inhibition of the three medicinal plants at the Belvedere was 0.33 (± SD 0.43). At the Berkeley Gump Station, the average inhibition was 0.53 (± SD 0.56). At Mari Mari Kellum's property, the average inhibition was 0.59 (± SD 0.79).

Yeast inhibition was not significantly affected by the roots of *Hibiscus rosa-sinensis,* compared to *Hibiscus tiliaceus* or *Thespesia populnea.* There was a significant difference between the positive control and all the treatments (ANOVA, $F_{4,100} = 40.59$, $p < 0.001$, Fig. 1). The positive control, negative control *H. rosa-sinensis*, *H. tiliaceus*, and *T. populnea* had a mean inhibition of 2.6, 0.43, 0.50, 0.40 and 0.67 (±SD 0.43, 043, 0.62, 0.78) respectively.

Yeast inhibition was not significantly affected by the aboveground parts of *Hibiscus rosa-sinensis,* compared to *Hibiscus tiliaceus* or *Thespesia populnea.* There was a significant difference between the positive control and all the treatments (ANOVA, $F_{4,50} = 94.1$, $p < 0.001$, Fig. 2). *Thespesia populnea* had the highest mean inhibition followed by *H. tiliaceus* and then *H. rosa-sinensis*, however these were not significantly different. The positive control, negative control, *H. rosa-sinensis*, *H. tiliaceus* and *T. populnea* had a mean inhibition of 22.58, 0.43, 0.15, 0.70 and 0.31 (± SD 0.43, 0.43, 0.18, 0.40 and 0.34) respectively.

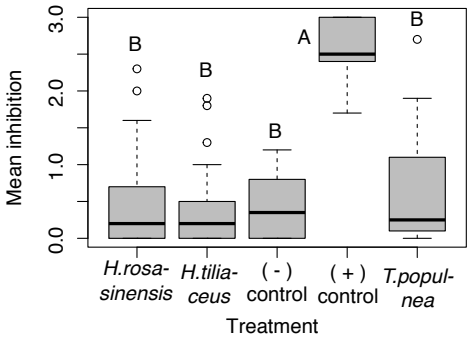


FIG. 1 Boxplot with quartiles and outliers (circles) of the belowground mean inhibition of each species in comparison to the controls. The only significant difference was between the positive control and all the treatments (ANOVA, $F_{4,100} = 40.59$, $p < 0.001$). Letters refer to a statistically homogenous groups (Tukey test).

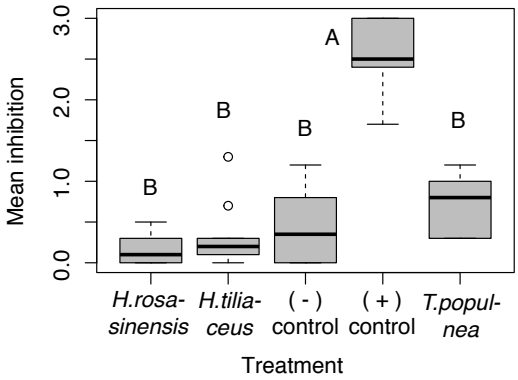


FIG. 2 Boxplot with quartiles and outliers (circles) Aboveground average inhibition of each species in comparison to the controls. The only significant difference was between the positive control and all the treatments (ANOVA, $F_{4,50} = 31.24$, $p < 0.01$). Letters refer to a statistically homogenous groups (Tukey test).

Yeast inhibition was not significantly affected by the belowground and aboveground parts of *T. populnea.* The positive control was significantly different from all the treatments (ANOVA, $F_{4,50} = 31.24$, $p < 0.01$, Fig. 3 ). The fruits of *T. populnea* had the highest mean inhibition followed by the roots and then the leaves. However, these parts were not significantly different from each other and the negative control. The mean inhibition for the fruits, roots and leaves of T. *populnea* was 0.78,

0.67 and 0.62 (± SD 0.29, 0.79 and 0.41) respectively. The average inhibition of the negative and positive control was 0.0.43 and 2.58 (± SD 0.43 and 0.43) respectively.

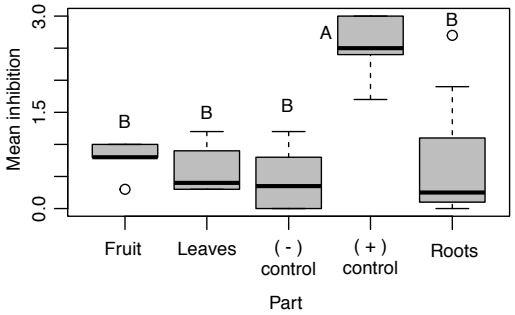


FIG. 3 Boxplot with quartiles and outliers (circles) of the mean inhibition of the belowground and aboveground parts of *T. populnea* in comparison to the controls. The only significant difference was between the positive control and all the treatments (ANOVA, $F_{4,50} = 31.24$, $p < 0.01$ ). Letters refer to a statistically homogenous group (Tukey test).

Yeast inhibition was not significantly affected by the belowground and aboveground parts of *H. rosa-sinensis.* The positive control was significantly different from all the treatments (ANOVA, $F_{4,60} = 52.69$, $p < 0.01$, Fig. 4 ). The roots of *H. rosa-sinensis* had the highest inhibition followed by the leaves and then the flowers. The mean inhibition of the roots, flowers and leaves was 0.50, 0.28 and 0.02 (± SD 0.62, 0.18 and 0.044) respectively. The average inhibition for the negative and positive control was 0.0.43 and 2.58 (± SD 0.43 and 0.43) respectively.

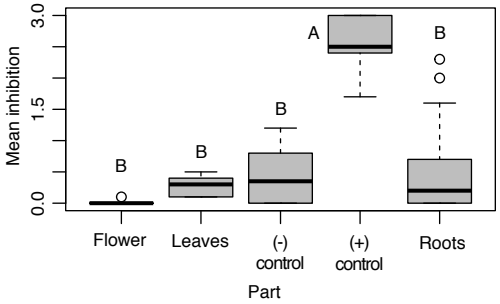


FIG. 4 Boxplot with quartiles and outliers (circles) of the mean inhibition of the belowground and aboveground parts of *H. rosa-sinensis* in comparison to the controls. The only significant difference was between the positive control and all the treatments (ANOVA, $F_{4,60} = 52.69$, $p < 0.01$). Letters refer to a statistically homogenous group (Tukey test).

Yeast inhibition was not significantly affected by the belowground and aboveground parts of *H. tiliaceus.* The positive control was significantly different from all the treatments (ANOVA, $F_{4,60} = 62.98$, $p < 0.01$, Fig. 5). The leaves of *H. tiliaceus* had the highest inhibition followed by the roots and then the flowers. The average inhibition for the leaves, roots and flowers of *H. tiliaceus* was 0.42, 0.40, 0.02 (± SD 0.57, 0.51 and 0.071) respectively. The average inhibition for the negative and positive control was 0.0.43 and 2.58 (± SD 0.43 and 0.43) respectively.

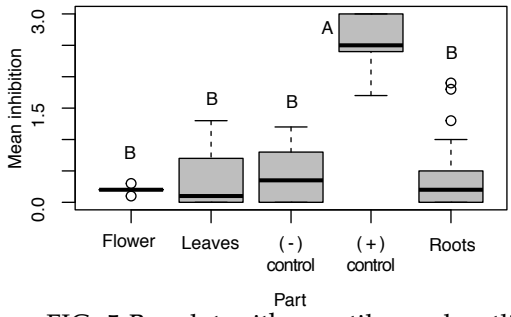


FIG. 5 Boxplot with quartiles and outliers (circles) of the mean inhibition of the belowground and aboveground parts of *H. tiliaceus* in comparison to the controls. The only significant difference was between the positive control and all the treatments (ANOVA, $F_{4,60} = 62.98$, $p < 0.01$). Letters refer to a statistically homogenous group (Tukey test).

*Soil nutrient content*

Nitrogen, phosphorus and potassium levels among the Berkeley Gump Station, Mari Mari Kellum's property and the Belvedere lookout were identical using the LaMotte NPK Soil Test Kit (data not shown). Nitrogen levels were below detection limits at all sites with less than 40 lb per acre for 6 inches of soil. Phosphorus levels were 8 lb per acre for 6 inches of soil and potassium levels were 160 lb per acre for 6 inches of soil at all three study sites.

*Decomposition of Belowground Biomass*

Only week one and three were significantly different (ANOVA, $F_{2,21} = 7.1$, $p < 0.01$) in root mass loss. Week one, two and three had an average root mass loss of 0.13,

0.17 and 0.22 (± SD 0.048, 0.047 and 0.045) respectively.

In comparing root mass loss among species, there was no significant difference (ANOVA< $F_{2,21} = 2.04$, p = 0.16, Fig. 6). *H. rosa-sinensis, H. tiliaceus* and *T. populnea* had an average root mass loss of 0.16, 0.20 and 0.15 (± SD 0.063, 0.047 and 0.058) respectively.

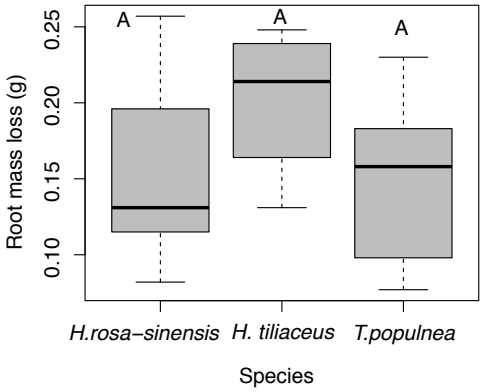


FIG 6. Boxplot with quartiles of the root mass loss of *H. rosa-sinensis, H. tiliaceus* and *T. populnea*. There was no significant difference for mass loss among species (ANOVA, $F_{2,21}$ = 2.04, p = 0.16). Letters refer to a statistically homogenous group (Tukey test).

There was a significant effect of week (ANOVA, F1,18= 18.04, p < 0.01, Fig. 7) and of species (ANOVA, F2,18= 3.59). However there was no interaction between week and species.

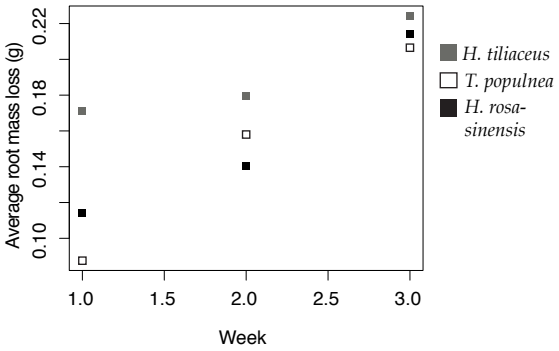


FIG 7. Average root mass loss of *H. rosa-sinensis, H. tiliaceus* and *T. populnea* by weeks. A fligner test was used to test for homogeneity of variances, as it was not significant a two-way ANOVA was used to test for differences between week and species. These results yielded a significant effect of week (ANOVA, F1,18= 18.04, p < 0.01) and of species (ANOVA, F2,18= 3.59). However there was no interaction between week and species.

There was a negative relationship between root decomposition rate and belowground antimicrobial activity, however it was not significantly different (Spearman rho = -0.56, n = 9, P = 0.12, Fig 8).

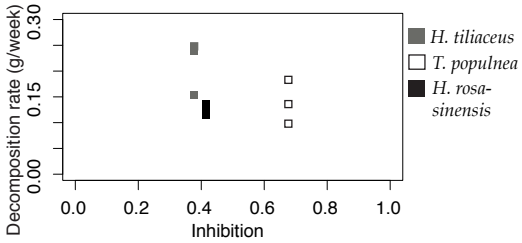


FIG 8. Root decomposition rate of *H. rosa-sinensis, H. tiliaceus* and *T. populnea*. There was no significant difference decomposition rate among species (Spearman rho = -0.56, n = 9, p = 0.12).

## DISCUSSION

The objectives of this study were to compare the belowground and aboveground antimicrobial activity in *H. rosa-sinensis, H. tiliaceus* and *T. populnea* and to determine whether the magnitude in belowground antimicrobial activity differed among species. This study also aimed to identify the correlation of belowground antimicrobial activity of the three species with root decomposition and soil nutrient content. This study showed that there was no difference in belowground and aboveground antimicrobial activity among species and in the aboveground and belowground parts of each species. This study suggested that belowground antimicrobial activity correlated with slower decomposition rates, but it was not statistically significant. This study was not able to conclude whether belowground antimicrobial activity correlated with soil nutrient content.

### *Belowground and aboveground antimicrobial assays*

To test belowground and aboveground antimicrobial activity, ethanol and water extracts were combined because they were not significantly different. Sites were also not significantly different and were therefore combined. The fruits of *T. populnea* had the highest mean inhibition followed by the roots and then the leaves. However the belowground and aboveground parts of *T. populnea* along with the negative control, were not significantly different. In *H. rosa-sinensis*, the mean inhibition was higher belowground than aboveground, but was not significantly

different. In *H. tiliaceus,* the leaves had the highest mean inhibition followed by the roots and then the flowers. Antimicrobial activity belowground and aboveground was also not significantly different.

In comparing the belowground antimicrobial activity among species, *T. populnea* had the highest mean inhibition followed by *H. rosa-sinensis* and then *H. tiliaceus*. However, all three species were not significantly different from each other or from the negative control. Thus this study did not support the evolution of introduced species hypothesis since native and introduced species had the same antimicrobial activity. As an aboriginally introduced plant (Whistler 1990), *H. rosa-sinensis*, might have escaped specialist herbivores from its native habitat and encountered new generalist herbivores (Hay and Morrison 2011) possibly causing it to allocate secondary compounds to plant defenses over the years

In analyzing the aboveground antimicrobial activity among species, there was no significant difference among species although *Thespesia populnea* showed a higher mean inhibition followed by *H. rosa-sinensis* and then *H. tiliaceus*. These results were not consistent with Cox's (2008) study that only focused on the aboveground parts. Cox's (2008) results showed that *H. rosa-sinensis* had the highest average inhibition followed by *T. populnea* and then *H. tiliaceus*.

The difference in results between Cox (2008) and this study on the aboveground antimicrobial activity may be due to half the use of replicates. While Cox (2008) used ten replicates per plant part, this study used five replicates per plant part due to time constraints. This experiment also focused more on using sterile materials to guarantee that the antimicrobial activity was because of the plant extracts alone. In addition, Cox (2008) measured yeast inhibition under the extract soaked filter paper, while this study measured the clear zone of growth inhibition around the filter discs. This methodology has been widely used in anti-microbial assays and has proven to be the most effective as shown in studies by Khoo et al. (2011) that evaluated the antimicrobial activity of different plant extracts. Lastly, it was not clear what strategy Cox (2008) used to assign a rating of 0-3 for yeast inhibition and therefore the perceptions of low, medium or high inhibition varied between both studies. Future studies can standardize the low, medium and high inhibition categories by using a ImageJ to measure the diameter of the zone of inhibition for each disc and assigning length values to each category as done by Frey and Meyers (2010).

In this study, the plant extracts had a low mean inhibition on baking yeast. Since previous studies conducted by Priya and Sejal (2012) showed that the roots of *H. rosa-sinensis* had the highest growth inhibition of *C. albicans*, *E. coli*, *S. aureus*, *S. pyogenes*, *C. albicans* and *P. aeruginoa* followed by the leaves and then the flowers, future studies should test for inhibition of yeasts that more commonly affect humans such as *Candida albicans*. Furthermore, studies can be expanded to compare the belowground and aboveground anti-microbial, anti-fungal, anti-bacterial or anti-viral properties of *H. rosa-sinensis, H. tiliaceus* and *T. populnea* since these have been identified to exhibit such inhibition (Anonymous 1998, Kumar et al. 2008, Kumari et al. 2008, Priya and Sejal 2012).

*Soil nutrient content*

In analyzing the soil chemistry at the Berkeley Gump Station, Mari Mari Kellum's property and the Belvedere lookout, there was no difference in soil composition among the sites. Nitrogen levels were below detection limits at all sites with less than 40lb per acre per six inches of soil. Phosphorus levels were 40lb per acre per six inches of soil and potassium levels were 160lb per acre per six inches of soil. As a result, the correlation between soil nutrient content and antimicrobial activity was inconclusive because the nitrogen, phosphorus and potassium levels were identical across all study sites. However, since soil nutrient content was identical at all sites and the belowground antimicrobial activity was not significantly different among species, it is possible that these two correlated. To further investigate this relationship, future studies can grow these medicinal plants in manipulated soils that have been enriched with varying nutrient levels and correlate the soil nutrient content with the antimicrobial activity. There is also a natural approach where future studies can search for sites throughout Moorea that are habitats for these medicinal plants and have varying soil nutrient levels.

The lack of diverse nutrient content also suggested that elevation did not seem to have an effect on the nitrogen, phosphorus and potassium levels, as these nutrient levels were the same coastal and mid-elevation

regions of the island. However, more soil nutrient testing needs to be done along an elevational gradient to determine whether elevation plays a role in soil nutrient composition.

The LaMotte NPK Soil Test Kit used in this study was not precise in measuring exact nitrogen, phosphorus and potassium levels since the NPK chart consisted of only three graphic color options of low, medium and high. Future studies can also use a different instrument to measure nutrient levels at a finer scale.

Ultimately, the lack of soil nutrients may be because the Society Islands of French Polynesia were formed from the same basaltic lavas (Dostal et al. 1982) of hot spot volcanoes (Segar and Segar 2007). Since basalt from volcanic islands contained other soil nutrients such as calcium, magnesium, iron and manganese (Eggleton et al. 1987) that are essential for plant growth (Silva & Uchida 2000), by measuring these different nutrient levels, there could have been a difference in soil composition. However, only nitrogen, phosphorus and potassium levels were measured due to limited lab equipment. Future studies can also compare the antimicrobial activity of the same medicinal plant species from island and mainland habitats.

### *Decomposition of Belowground Biomass*

In analyzing decomposition of belowground biomass, within the first week *H. tiliaceus* decomposed faster than the other species, but by week three, there was no significant difference in root mass loss among *H. rosa-sinensis, H. tiliaceus* and *T. populnea (*Figure 6*).* However, there was a positive relationship between root mass loss and time in weeks, where the only significant difference was between weeks one and three. Root mass loss among species was significantly different when all three time points were analyzed simultaneously.

Root decomposition rate and antimicrobial activity showed a negative relationship, however it was not significant. Nonetheless, this suggested that the roots of the three species had the same secondary compounds, as secondary compounds are also directly related to the rate of decomposition (Paul 2006).

### *Conclusion*

Overall, as the majority of antimicrobial studies on medicinal plants have focused on the aboveground parts alone of mainland medicinal plants, this study was unique in that it demonstrated that belowground and aboveground antimicrobial activity did not differ among and in each of three plant species of the same family found in the island of Moorea. Thus, this study did not support the evolution of increased competitive ability hypothesis as native and introduced species had the same antimicrobial activity. Furthermore, because of the same antimicrobial activity at all sites for all species and the homogeneity of the soil nutrients, there was not enough evidence to support the growth-defense trade-off. Lastly, there was not enough evidence to correlate antimicrobial activity with a slower root decomposition rate. However, future studies should consider testing the antimicrobial activity of these medicinal plants on different yeast strains such as *C. albicans*, that are more relevant to human health, in a range of different soil types.

## ACKNOWLEDGMENTS

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**APPENDIX A**

| Plant | Leaves | Flowers | Roots | Fruit | Biological Activity |
|---|---|---|---|---|---|
| ***H. tiliaceus*** | fractured bones[4], sprained muscles[4], postpartum discharge[4], delivery[4], coughs[4], sore throat[4], open wounds[4,] fevers[7], ulcers[7], skin diseases[7] | sores[4], cuts[4], boils[4], swellings[4] | dysentery[8], microbial infection[8], skin boils[8], chest congestion[8] | No information | antioxidant[8], microbial infection[8] |
| ***H.rosa-sinensis*** | postpartum relapse sickness[4], boils[4], sores[4], inflammations[4], laxative emollient[6], emmena-gogue[6], aphrodisiac[6] | menstruation[2], cystitis[2], venereal diseases[2], feverish illnesses[2], carbuncles[2], mumps, sores[2], coughs[2], gonorrhea[6] | coughs[6], gonorrhea[6], menorrhagia[6] | N/A | Antioestrogenic[4], anti-implantation[4], abortifacient[4], antipyretic[4], antispasmodic[4], CNS depressant[4], hypotensive[4], antispermatogenic[4], embryotoxic[4], hypothermic, insect attractant[4] analgesic[4], antifungal[4], and anti-inflammatory[9]. |
| ***T. Populnea*** | coughs[4], influenza[4], headache[4], relapses in illnesses[4], cutaneous infections[1] | astringent[5], hepatoprote-ctive[5], antioxidant[5], cutaneous infections[1] | dysentery[3], cholera[3], hemorrhoids[3] | urinary tract problems[4], abdominal swellings[4] dysentery[1], cholera[1], hemorrhoids[1] cutaneous infections[1] | antibacterial[4], antifungal[4], antiyeast[4], anti-implantation[4], antispasmodic[4] |


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