

COMPARISON OF ANTIFUNGAL ACTIVITY BETWEEN PURE EXTRACTS AND MIXTURES OF *FICUS BENGHALENSIS* LEAVES AND *CURCUMA LONGA* RHIZOMES

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Abstract. Antifungal activity of pure extracts and mixtures with coconut milk or coconut water of *Curcuma longa* and *Ficus benghalensis* were tested against *Saccharomyces cerevisiae*. Results from the antifungal bioassay revealed that neither *Curcuma longa* nor *Ficus benghalensis* had an effect against *Saccharomyces cerevisiae*. This goes against what was discovered through interview with experts and healers in Moorea and the published literature. Interviews also yielded the medicinal use of “uto” in Tahitian medicine along with a list of plants that are now difficult to find Moorea, French Polynesia. Transects confirmed that except for *Syzygium malaccense*, none of the rare plants mentioned by the healer could be found in the wild.

Key words: *ethnobotany; antifungal plants; medicinal mixtures; Cocos nucifera; Curcuma longa; Ficus benghalensis*

INTRODUCTION

Ethnobotany is a study that focuses on how people of a particular culture understand and use medicinal plants to treat illnesses (Cox and Banack 1991). Researches in ethnobotany have led to the discovery of compounds that are commercially successful such as aspirin from *Filipendula ulmaria* and pseudoephedrine from *Ephedra sinica* (Cox and Balick 1994). Taxol a drug derived from *Taxus brevifolia* and used in cancer treatments accounts for nearly \$2 billion in revenue for the pharmaceutical company that makes it (Mann 2002). The intense focus on medicinal plants has led to the fear that some plants may be threatened. Ethnobotany in areas such as the South Pacific is roughly divided into three time periods. The first is a period of observations made by some of the first explorers and naturalists who landed on islands in the South Pacific (Merlin 2000). The second period is that of “resident-naturalists” such as missionaries and physicians who settled in these islands and recorded the medicinal uses of plants. Ethnobotany in its present form falls into the third category. Ethnobotany at this time uses a more

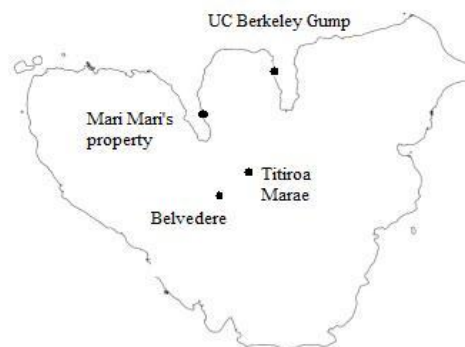


FIG. 1. Map detailing transect sites in Moorea, French Polynesia. Map courtesy of the Geospatial Innovation Facility, University of California, Berkeley.

standardized way of studying cultural use of plants using methods from both anthropology and botany to identify and categorize medicinal plants (Merlin 2000).

Traditional Tahitian medicine or *Raau Tahiti* is divided into 4 categories *raau hea*, *raau ira*, *raau fati*, and *raau vitae* (Gaertner 1994, H. Murphy, pers.comm.). *Raau hea* is used to “purge the body”, *raau ira* is used as “preventative or curative medications for convulsions in children” and *raau fati* is used for “fractions, contusions, and broken bones”

(Gaertner 1994). *Raau vitae* is the most closely guarded illness category. *Raau vitae* deals with spiritual cleansing that rid the body of curses. Healers prepare different medicinal mixtures for each patient depending on the illness. Plants knowledge that is use to treat common illnesses is shared among everyone but herbal mixtures for more complicated illnesses are kept a family secret. Specific conditions such as Type II Diabetes are treated using *Morinda citrifolia*, *Casuarina equisetifolia*, and *Persea americana*, and *Cymbopogon nardus* but their exact method of preparation is not known (Glaser 2001). One reason why healers do not want to divulge specific information about their medicine is to prevent people from misusing the plant or to protect the location where rare plants are found (V. Brotherson, pers. comm.). Coupled with the secrecy of Tahitian medicine and the limited published literature on French Polynesian ethnobotany, a study on plant use in Moorea will add to the knowledge of ethnobotany in the South Pacific.

Moorea serves as an interesting study site for ethnobotany because it is heavily impacted by plant introductions and human activity. Published books on French Polynesian ethnobotany is limited and for this reason, healers and experts till this day still reference Paul Petard's book on Tahitian traditional medicine. Previous ethnobotanical research in Moorea yielded interesting results. Tahitian medicine also includes the use of lichens, ferns, and even a particular species of algae that treat various medicinal conditions (Holl 2002; Vassef 2002; Baltrushes 2005). Antifungal bioassays have been used to test for a plant's medicinal properties. Earlier antifungal assays showed inconclusive results but the methods have been improved over the years to be more sensitive to the treatments (Achrekar 1995; Holl 2002; Cox 2008). Trotter in 2005 could not get any conclusive result from her antifungal bioassays for *Geophila repens* and *Centella asiatica* despite reports that they have antifungal properties. Cox was the first student to get positive results from her antifungal assay that shows an inverse relationship between antifungal activity and herbivory between *Thespesia populnea*, *Hibiscus tiliaceus*, and *Hibiscus rosa-sinensis*. This led to

many adaptations on Cox's methods by other students (Chan 2009; Yang 2010).

Fungal skin infections are common and typically affect moist areas of the body such as between the "fingers, toes, under breasts, and in the genital area" (Stevens 2000). Symptoms of a fungal infection include scaling of the skin, redness, and swelling (Stevens 2000). The humid climate of Moorea is a favorable condition to foster fungal growth on skin. Previous studies have overlooked the fact that the majority of Tahitian medicine is given to the patient as a mixture with coconut water or coconut milk as a liquid medium (Cox and Banack 1991; H. Murphy pers. comm.). Among the antifungal plants that Tahitians use to treat fungal skin infections includes *Alphitonia zyziphoides*, *Hibiscus tiliaceus*, *Sapondias mombin*, and *Hedyotis romanzoffiensis* (Bertrand *et al.* 2013). However, some of these plants are difficult to find. A common Tahitian remedy use to treat fungal infection involves grating turmeric roots (*Curcuma longa*) and mixing it with coconut milk (H. Murphy, pers. comm.). The mixture is applied to the skin for three days. Another plant that is used is banyan leaves or "ora" in Tahitian. Water from boiled banyan leaves (*Ficus benghalensis*) is applied to the skin to dry it out (J. You-Sing, pers. comm.). Banyan leaves are used as preventative medicine, to treat cancer, and fungal skin infections (Gaertner 1994; J. You-Sing, pers. comm.).

The purpose of this paper is to compare the results from antifungal tests between pure extracts of turmeric roots or banyan leaves and when the plant is in a coconut water or coconut milk mixture. I predict that the mixture will have higher antifungal activity than the pure plant extract since mixtures contain several plants that are reported to have beneficial use. Interviews were conducted with experts and healers to get a better sense of antifungal plants use in Tahitian medicine and to get an understanding of plants that are now declining. Transects were performed to see if any of the plants the healer mentioned as rare could be found in the wild. Antifungal activity against *Saccharomyces cerevisiae* was assessed using the disc diffusion method and were adapted from previous studies (Cox 2008;

Yang 2010). If the extract showed any sort of ability to inhibit yeast growth, then there will be a translucent ring around the filter disc. The zone of inhibition is defined as the diameter of the translucent circle that includes the filter paper and “indicate the extent of the test organism’s inability to survive in the presence of the test antibiotic” (“Examples of Antibiotic Sensitivity Testing Methods”). The larger the zone of inhibition, the more effective the treatment is at killing the yeast.

METHODS

Study site

This study was done in Moorea, French Polynesia (17.53°S, 149.83°W). Transects were performed at Mari Mari Kellum’s coastline property (17°30′51.34″S, 149°50′53.42″W), the Belvedere lookout (17°32′25.79″S, 149°49′36.08″W), and Titiroa Marae (-17°32′12.0402″, -149°49′47.2182″) (Fig. 1).

Interviews

One interview with healer Mrs. Jean You-Sing was conducted at her home with the help of Valentine Brotherson who acted as a translator. Questions were asked to get a better understanding of the healer’s perception of traditional medicine and how she has been affected by the decline of certain plants. Three interviews with Hinano Murphy, associate director at the UC Berkeley Gump Station and an expert in Tahitian medicine, were held at the Gump Station. The names of medicinal plants were given in Tahitian Paul Petard’s book *Raau Tahiti* was consulted to find the equivalent scientific name for the plant. Any scientific name changes since the publishing of Petard’s book was accounted for. Vouchers were not made for ora leaves or turmeric roots because ora leaves were too large and turmeric roots had to be preserved.

Questions asked during the interviews:

1. Have you noticed any changes (environmentally or socially) in traditional medicine in Moorea?

2. Have you noticed the decline of certain medicinal plants?
3. Are you aware of any conservation efforts to protect the future of these important plants?
4. Are you having any difficulties finding certain plants and how has this affected the way you are preparing your medicine?

Agar Plates and Yeast Preparation

Methods for the antifungal bioassays were modeled after those in Cox 2008 and Yang 2010 with some modifications of my own. Only fresh turmeric roots and banyan leaves were used for the experiment. Fresh turmeric roots were purchased from Papeete Municipal Market in Tahiti, French Polynesia because none were found at grocery stores in Moorea or in the wild. Banyan leaves were collected from either in the neighborhood near the ferry terminal in Tahiti or from the Opunohu area. Local coconut milk was purchased from a grocery store in Moorea and fresh coconut juice was collected from a green coconut collected from the Atitia Center, Moorea.

An agar mixture was created using 5 grams of gum agar powder, 35 grams of white granulated sugar and 350 mL of deionized water. The agar mixture was autoclaved at 121°C and 0 psi for 15 minutes to sterilize it. After sterilizing it, the agar beaker was allowed to cool and then transferred immediately to a fume hood. A thin film of agar was poured into each sterile Petri plates that measured 100 mm in diameter and 15 mm in height. After pouring the agar, the Petri dishes were covered, inverted and stored for 24 hours until the agar solidified. 3 grams of baker’s yeast (*Saccharomyces cerevisiae*) was combined with 30 mL of deionized water. Baker’s yeast was used for its availability. Sterile cotton swabs were dipped into the yeast solution and then spread evenly onto the agar in a zigzag formation. This ensured that the entire surface of the agar was evenly covered with a thin layer of yeast. After spreading the yeast, the Petri dishes were covered and wrapped with Parafilm on the edges. The dishes are then inverted and the

TABLE 1. Abbreviations of pure extracts, mixtures, and controls.

Pure Extracts	Mixtures	Controls
Cu(OH) = curcuma – 90% ethanol	CCW = curcuma-coconut water	Deionized water
Cu(H ₂ O) = curcuma-deionized water	CCM = curcuma-coconut milk	1% econazole nitrate (antifungal cream) + 90% ethanol
Ora(OH) = ora-90% ethanol	OCW= ora-coconut water	Coconut water
Ora(H ₂ O) = ora-deionized water	OCM = ora-coconut	Coconut milk
		90% ethanol

yeast is allowed to grow for 24 hours in an incubator kept constant at 33°C.

Extracts and Mixtures

5 grams of fresh plant materials (turmeric roots or banyan leaves) were wiped with a KimWipe dipped in 90% ethanol and sliced into thin pieces using a razor. The plant materials is then blended with 30 mL of 90% ethanol, deionized water, coconut milk, or coconut water in a kitchen blender until it is homogeneous as possible. The resulting liquid was transferred into vials using a pipette. Vials with ethanol extracts were refrigerated for 3 days before use. Each vial was shaken daily three times a day for 3 days. Water extractions and coconut milk/coconut water mixtures could be used immediately. Ethanol extractions and water extractions were performed because previous studies have shown that solvent type and concentration may alter the result, depending on the solubility of the plant compounds (Wendakoon *et al.* 2012). The positive control is the antifungal cream (1% econazole nitrate) mixed with 90% ethanol and the negative control is deionized water, 90% ethanol and coconut water and coconut milk. In addition to the extracts and mixtures, 2 banyan leaves and turmeric roots were boiled separately to test for antifungal properties. The recipe given by the healer calls for 50 banyan leaves boiled in 2 liters of water. Unfortunately, this could not be done and the amount was scaled down to 2 banyan leaves (or 2 turmeric roots) in 50 mL of water.

A hole-puncher was used to create small filter paper discs that measures 0.4 cm in

diameter. The hole-punched filter paper discs were placed into a beaker and covered with aluminum foil. The beaker containing the filter paper discs was autoclaved at 120°C and 0 psi for 15 minutes. This ensures that the filter paper discs are sterilized prior to use. Working underneath a fume hood, filter paper discs were removed from the beaker using sterilized tweezers and then dipped 3 times into a vial containing one of the treatments. The filter disc was then placed onto the yeast that was allowed to grow for 24 hours. Approximately 6 filter paper disc dipped in different treatments were placed in the same Petri dish. This was done to vary the conditions for the treatments. If there was contamination in one agar plate, then at least the other agar might show some results. 20 replicates of each treatment were performed. The plates were incubated in the same incubator at 33°C for 3 days. Presence or absence of antifungal activities was recorded after 3 days. Digital photographs of the Petri plates were taken next to a ruler.

Transects

The purpose of the transects was to see if any of the plants the healer mentioned could be found in the wild. One transects measuring 25 meters in length was performed at each of the three sites: Belvedere lookout, Mari Mari's coastline property, and Titiroa Marae. Areas were chosen at random and the following plants were noted if there was any: turmeric, ora, coconut (tall or dwarf variety), *Oxalis corniculata*, *Leucas decedentata*, *Ophioglossum pendulum*, *Persicaria glabra*, and *Syzygium malaccense*. Presence of any of these plants

within one meter of either side of the transect tape at each meter was recorded.

Statistical Analysis

No statistical analysis was done for the transect data because it was simply to see whether or not a plant could be found. Photographs of the Petri dishes were used to measure the zone of inhibition using ImageJ (Rasband1997-2012). Data from the antifungal bioassay were analyzed using R-Studio (R Development Core Team 2013). A Glht test (general linear hypothesis test for multiple comparisons of means) was performed. The result from the Glht gives the relationships between all the different treatments and whether or not they are significant. A significant relationship would have a P-value of less than 0.05. All relationships with a P-value greater than 0.05 is insignificant.

RESULTS

Interviews

Although Mrs. You-Sing specializes in treating breast cancer, she is also consulted for other illnesses. Mrs. You-Sing echoed what was already reported in previous papers when asked if she has noticed any changes in Tahitian medicine in the recent years. She notes that Tahitian medicine is on the decline because the younger generation does not seem to be interested in traditional medicine. She also states that many of the important plants

that are used in Tahitian medicine are now very difficult to find and it is impossible for her to substitute any of these plants with another plant. Some of these plants include *Oxalis corniculata*, *Leucas decemdentata*, *Ophioglossum pendulum*, *Persicaria glabra*, and *Syzygium malaccense*. Some of these plants grown for personal use and many people are not willing to share. Some of these plants could be found at the Atitia Center.

The medicinal uses of coconut goes far beyond using the juice or milk. There is only one species of coconut on Moorea, which is *Cocos nucifera* but healers can distinguish between many different varieties. The two varieties that are easily distinguishable by everyone are the dwarf coconut and tall/wild coconut. Dwarf coconuts were cultivated to be smaller than their wild counterparts and are planted as ornamental plants around homes (H. Murphy, pers. comm.). Some healers refuse to use the dwarf coconuts because they believe that cultivation has caused the coconut to have less medicinal properties than the wild coconuts. One of the components in Mrs. You-Sing's mixtures for treating breast cancer is the "uto" of a sprouting coconut. The "uto" or "coconut apple" is a developing cotyledon that eventually absorbs the endosperm of a coconut (Armstrong 2003). It is edible by itself and can be used in medicinal mixtures as well. The uto can also be used to "regenerate the skin" (H. Murphy, pers. comm.).

Antimicrobial bioassays

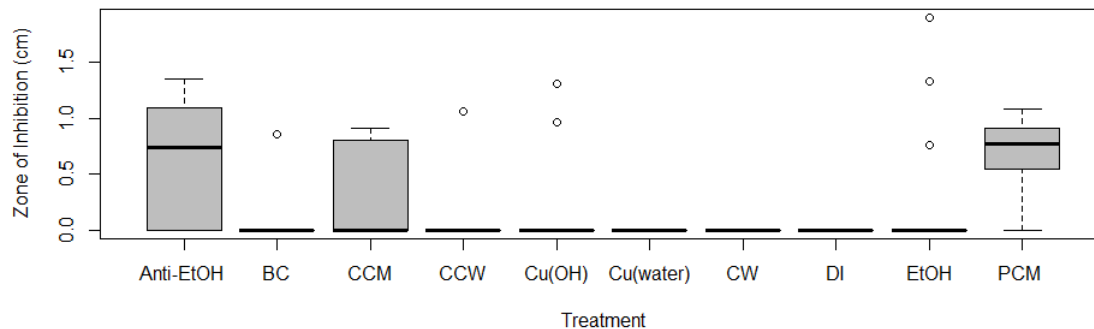


FIG. 1: Inhibition zone of pure turmeric extracts and mixtures of turmeric with coconut water or coconut milk. Please refer to Table 1 for explanation of the abbreviations

With the exception of some outliers, none of the water or ethanol extracts along with the boiled treatments showed any fungal inhibition (Fig. 1, Fig. 2). The positive control (Anti-EtOH), negative control (PCM) and mixture with coconut milk were the only treatments that showed consistent fungal inhibition. For both of the graphs, the inhibition zone between PCM and Anti-EtOH did not differ significantly (Glht, $P=1.0$). Both graphs suggest that neither turmeric nor ora leaves have antifungal activity. Yeast inhibition differs significantly between treatments (Kruskal-wallis, $\chi^2 = 97.6$, $df = 9$, $p < 2.2e-16$) (Fig 1). As with Figure 1, Figure 2 also showed that yeast inhibition differs significantly between treatments (Kruskal-wallis, $\chi^2 = 85.1$, $df = 9$, $p < 1.6e-14$). All treatments were significantly lower than the positive control, Anti-EtOH, with the exception of PCM and CCM (Glht, $p < 0.001$). Relationships with PCM and other treatments are significant (Glht, $p < 0.01$, with exception of PCM and CCM (Glht, $p = 0.04$) (Fig. 1). Results for Figure 2 is similar to that of Figure 1. Similarly, Anti-EtOH, OCM and PCM were significantly different from all other treatments but are insignificant when compared to one another. The pairwise relationship of OCM and Anti-EtOH (Glht, $p = 0.30$) shows that these two relationships are not significant (Fig. 2).

Transect data

Among the studied plants, the most abundant plants were dwarf coconuts and *Syzygium malaccense*. These two plants are found independently of each other. *Syzygium malaccense* is found more inland, such as the Belvedere and Marae sites whereas the coconut that were encountered are closer to the coast, such as those at Mari Mari's. *Leucas decemdentata* was encountered at the Atitia Center where it is cultivated for personal use. *Persicaria dichotoma* and *Ophioglossum spp.* was not seen anywhere.

DISCUSSION

Interviews

The decline of Tahitian medicine mentioned in the interviews with Mrs. You-Sing and Hinano Murphy is in line with what previous studies have reported (Gaertner 1994; Trotter 2005). Mrs. You-Sing repeatedly stated that she is unable to substitute any of the rare plants in her mixtures. The disappearance of medicinal plants coupled with the younger generation's lack of enthusiasm for Tahitian medicine may lead to a loss of medicinal knowledge. Up until recently, healers did not record their medicinal knowledge down because much of it is passed verbally between the female relatives (V. Brotherson, pers. comm.). Thus, many people, including Mrs. You-Sing herself, reference Paul Petard's book on Tahitian medicine. Conserving medicinal knowledge in

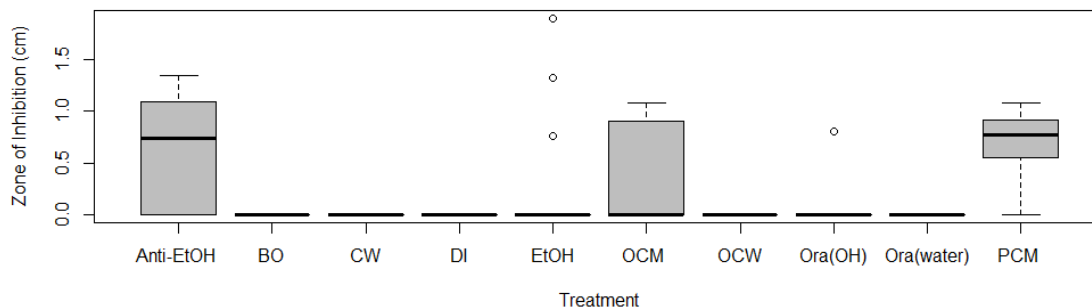


FIG. 2: Inhibition zone of pure ora extracts and mixtures of ora with coconut water or coconut milk. Please refer to Table 1 for explanation of the abbreviations.

the form of writing is just as important as protecting the rare plants. The Atitia Center is a cultural center located on Gump property that serves as an area of gathering and cultural learning. It also maintains a number of medicinal plants including some of the rare plants that Mrs. You-Sing mentioned.

Antimicrobial assay

The hypothesis is rejected because although the curcuma and coconut milk mixture showed antifungal inhibition, it is possible the coconut milk in the mixture have antifungal properties. The pure coconut milk control (PCM) was one of the three treatments that showed any activity. There are various reasons why the extracts did not show any inhibition, including the possibility that the extracts and mixtures have to be purified before it could be used. However, procedures to purify something would require complicated procedures and equipments. Another factor that could have affected the antifungal activity of the boiled ora is that I did not follow the healer's recipe exactly. Perhaps the concentration of ora leaves to water could have affected the result. There is also the possibility that some contaminants were introduced throughout the process. Although most of the experiment was done under a fume hood to minimize contamination, there were other opportunities for contamination. 90% ethanol may not be the ideal solvent for crude extraction of turmeric and banyan leaves.

Transect

Findings from the transects were not surprising. The rarity of *Ophioglossum spp.* is expected since it was mentioned in Gaertner (1994) that it could only be found in government-owned botanical gardens. It was surprising that I could not find any turmeric plants in the wild or at the supermarket. The closest member I found to the turmeric is ginger (*Zingiber officinalis*) which was abundant. Turmeric could be cultivated in personal gardens.

Future Study

Future studies could focus on how the concentration of individual plants in a mixture affects antifungal activity. The minimal inhibition concentration of each plant could also be recorded and compared with the antifungal results to see if there is a correlation. One interesting observation that was noticed but not recorded was the antifungal activity of several treatments after the three day limit. Students could also test when the first sign of yeast inhibition occurs and how this changes over time for several treatments.

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