Examining the UV-Absorbing Properties of Scaevola taccada (Goodeniaceae) and Its Potential Use as a Sunscreen

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Examining the UV-Absorbing Properties of *Scaevola taccada* (Goodeniaceae) and its Potential Use as a Sunscreen[1]

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Abstract: The native Hawaiian plant, *Scaevola taccada*, commonly grows in tropical coastal areas throughout the Pacific and Indian oceans. Oral tradition suggests that the fruit of this species may have been used as a natural sunblock. To examine this, an extract of *S. taccada* fruit was tested to determine whether it had UV-absorbing properties, and if so, to determine its stability and potential toxicity to coral. *Scaevola taccada* contained UV-absorbing compound(s) with a sun-protection factor of 20, on average, that absorbed in the UVB and UVA (19.6% and 24.4% absorbance, respectively). The UV stability of the fruit extract over time and to various treatments (fresh, heated, and frozen) did not change (ANOVA, $p = .396$, $F = 1.018$). In addition, we examined the toxicity of *S. taccada* juice by exposing coral fragments of *Porites compressa* ($n = 7$) to four treatments (fresh and heated *S. taccada* extract, cytotoxic *Montipora capitata* eggs, and filtered seawater control). Changes in color and photosynthetic yield in treated tissue areas were measured after 4 days. Only the concentrated *S. taccada* fruit extract and *M. capitata* eggs resulted in significant changes in pigmentation or photosynthetic yield of the coral ($p \leq .0001$). Further investigation is needed to isolate and expand tests on the UV-absorbing component(s) to understand this toxicity more clearly.

Keywords: native plant, corals, toxicity, sunscreen

Polynesians developed a regulated and sustainable natural resource management system long before this concept was recognized as important in Western culture (Johannes 1982, Abbott 1992). Today, there is growing interest in Hawai'i to revitalize traditional management practices and use traditional knowledge in concert with Western scientific approaches. Using traditional knowledge, we may find solutions that help mitigate the damage caused by modern sunscreens to coral reefs. Hawaiian oral history suggests that a native beach plant may contain UV-absorbing compounds that may be more eco-friendly. In that regard, we were interested in the oral tradition surrounding *Scaevola taccada* (Gaertn.) Roxb. and its use as a sunscreen.

Scaevola taccada (Hawaiian name, naupaka kahakai) is a coastal shrub indigenous to the Hawaiian Islands (Figure 1). This plant is highly tolerant to salt water and is commonly found on the seashores of tropical and subtropical areas in the Pacific Ocean and Indian Ocean (Howarth et al. 2003, Bornhorst 2005). Traditionally, Hawaiians used *S. taccada* for its antibacterial and antiviral properties, and it has been used to treat skin disorders, wounds, and cuts (Ka'aiakamanu 2003, Gutmanis 2017). In addition to these medicinal properties, it has been suggested by some

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FIGURE 1. *Scaevola taccada* (Gaertn.) Roxb. Naupaka kahakai, or *Scaevola taccada*, is a coastal shrub indigenous to the Hawaiian Islands. This plant thrives in salt spray and commonly grows on open sandy beaches throughout the Indo-Pacific. The smooth, fleshy fruits turn white as they mature (*A*, unripe; *B*, intermediate; *C*, ripe). The mature white fruits have a spongy texture that helps them float in seawater. Photo: K. Rochette-Yu Tsuen, Kapiʻolani Community College, Honolulu, HI.

local ethnobotanists that *S. taccada* may have been used traditionally as a natural sunblock. Some have speculated that *S. taccada* fruits contain the protective compound while others argue that it is located in the leaves (personal communication, M. K. Keliipuleole). However, no previous research had tested the plant's UV-absorbing ability. The goal of this paper was to understand the basis of this traditional oral history by examining whether the fruits of *S. taccada* have any UV-absorbing properties. Additionally, we were interested in how this fruit extract, if used as a sunscreen, might interact with the marine environment.

It is widely accepted that coral reefs are in decline worldwide due to global and local causes (Wilkinson 2000). Globally, ocean acidification and warming cause stress and increased bleaching in corals (McClanahan et al. 2007, Leggat et al. 2019). Locally, sources of pollution, sedimentation, and overfishing are contributing to the decline of coral colonies (Stuhldreier et al. 2015). Another potential threat that has been discussed in the media recently is whether current types of sunscreen might also be a contributory source of local pollution on reefs (Downs et al. 2016, DiNardo and Downs 2018).

A major component of many commercial sunscreens is oxybenzone. Oxybenzone is an efficient organic UV filter widely used in pharmacology and cosmetology to provide protection against harmful UV radiation: UVB (280–315 nm) and UVA (315–400 nm) (Gupta 2013). Due to their high kinetic energy, these wavelengths have the ability to

damage DNA molecules, increasing the risks of cancer after prolonged and repeated exposure to sun's rays (Kielbassa et al. 1997). Oxybenzone has been proven to interfere with coral larval settlement, growth, and recruitment (Downs et al. 2016). It has also been associated with several small-scale bleaching events (DiNardo and Downs 2018). In addition to its negative impact on coral ecosystems, oxybenzone has been identified as a persistent endocrine disruptor in humans (DiNardo and Downs 2018). This compound has raised concerns worldwide and recently the State of Hawai'i passed a bill (SB 2571) to ban sunscreens containing oxybenzone from the local market as of January 2021. In this study, we tested whether *S. taccada* fruit extracts had UV-absorbing properties and thus the potential to be a "reef safe" sunscreen. To that end, a second goal of this paper was to test whether *S. taccada extract* was toxic to coral tissue.

MATERIALS AND METHODS

We examined the UV characteristics and UV stability of *S. taccada* fruit extract over time in fresh, heated, and frozen preparations with a spectrophotometer. Additionally, we tested the toxicity of this extract on the tissue of coral colonies (*Porites compressa*). Treatments included agar-filled caps attached to coral fragments over a period of 4 days. These agar-filled caps were made with filtered seawater (positive control), fresh and concentrated *S. taccada* extract, and crushed *Montipora capitata* eggs (negative control), which has proven to be toxic to coral tissue (Hagedorn et al. 2015). The responses of the coral to these treatments were measured over time with microscopic visualization paired with image analysis and viability assessment of the symbionts' photosynthetic yield in the treated areas with a Pulse Amplitude Fluorometer.

Fruit Collection and Extraction

The ripe, drupaceous fruits of *S. taccada* were collected at He'eia State Park and the University of Hawai'i at Mānoa on the island of O'ahu. An extract was made by squeezing the fruit through muslin cloth. The filtrate was collected in a 15 mL centrifuge tube and centrifuged at 7,000 relative centrifugal force (rcf) for 5 to 7 min in order to pellet the debris. The supernatant of the extract was transferred to a clean 15 mL centrifuge tube.

Coral Collection

Porites compressa colonies were collected around Moku o Lo'e (Coconut Island) in Kāne'ohe, Hawai'i during summer 2019 and maintained in flowing seawater tables at the Hawai'i Institute of Marine Biology (HIMB), University of Hawai'i. Coral collection was performed with appropriate permits from the state of Hawai'i's Department of Land and Natural Resources (Special Activity Permit SAP #2020-25). Coral fragments, approximately 10–15 cm in length with a relatively flat, Y-shaped profile, were used in the experiments.

Experiment 1—Assessing the Stability of S. taccada *Juice Treatments*

Extracts from 10 fruits were each divided into three volumes for three different treatments. To determine UV stability over time, fresh *S. taccada* extract was stored in a closed 2 mL Eppendorf centrifuge tube at room temperature (25 °C) and its absorbance was tested over time ($t = 0$, 1, 2, and 24 h). The second treatment determined the UV stability after the fresh fruit extract was heated. *Scaevola taccada* juice was aliquoted into 2 mL Eppendorf centrifuge tube and the absorbance of the extract was tested before the treatment. The extract was placed in a block heater at 60 °C, and its absorbance value was measured again after 10 and 60 min. A third treatment examined the UV stability of the frozen extract. *Scaevola taccada* extract was aliquoted into 2 mL Eppendorf centrifuge tube and frozen at −20 °C for at least 8 h. The extract was thawed, and the absorbance tested. Absorbance and sun protection factor assessment were evaluated following the method below.

Spectrophotometric Analysis: Absorbance and Sun Protection Factor (SPF) Values

UV irradiance can be categorized into UVA (400–315 nm), UVB (315–280 nm), and UVC (280–100 nm) (Gupta 2013). The absorbance of the *S. taccada* extract in the UV range was determined using a Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, Waltham, MA, USA) and the SPF was calculated from absorbance at wavelengths between 290 and 320 nm as determined by Gupta (2013). Briefly, 2 μL of *S. taccada* extract was pipetted on the pedestal of the Nanodrop and absorbance values were taken at interval of 5 nm to calculate an SPF for each set of treatments. To calculate the total area under the absorbance curve, the absorbance spectrum was estimated using uncircumscribed rectangles under the curve. From 200 to 700 nm, rectangles were traced every 5 nm (width) and absorbance was determined graphically (length). For values 700 to 800 nm, absorbance values were close enough to zero to be considered negligible.

Experiment 2—Assessing the Effects of S. taccada *Extract on the Viability of Live Symbionts and Tissue in P. compressa Fragments*

Porites compressa coral fragments were exposed to four different treatments: (1) filtered seawater (FSW, positive control); (2) frozen crushed eggs of *Montipora capitata* mixed with FSW (negative control), eggs were previously frozen at −80 °C, thawed at 30 °C, and mixed with FSW; (3) *S. taccada* extract; (4) *S. taccada* extract concentrated by heating at 60 °C for 30–40 min until the solution evaporated to one quarter of its original volume.

Gel Treatment Preparation for Cutaneous Application on P. compressa *Fragments*

To ensure that treatments were in close association with the coral tissue, agar disks containing each treatment were attached to the coral. A 1:3 ratio of 2% agar was mixed with each of the treatment solutions (v/v) resulting in a soft gel of ~0.5% agar. Caps of 2 mL Eppendorf centrifuge tubes were filled with the gel solution and left to harden at 4 °C for 5–10 min. Once the gels were hardened, the Eppendorf caps were glued to a zip tie. The four treatments were secured to each coral fragment ($n = 7$) and placed back in a flowing seawater table for 4 days. This methodology ensured that each coral fragment was in direct contact with each of the four treatments in the agar caps, but no dilution or decrease in the solution strength over time was assessed. After 4 days, the corals were assessed macroscopically and microscopically.

Photo and Colorimetry

A photo bucket was used to take consistent color pictures of the *P. compressa* fragments with uniform light. This process allowed us to accurately compare fragments before and after treatment and assess the damage from the treatments. The photos were taken using an Olympus Tough TG-4 camera (Olympus, Tokyo, Japan) and the Hawaiian Koʻa Card for color reference (Bahr et al. 2020). Computer-assisted photo analysis (NIH ImageJ) was used to measure changes in color in the treatment areas. The software generated a histogram of the coloration in the selected area, and this was converted into a black and white scale with values ranging from 0 (black) to 255 (white). Additionally, photos were taken through a dissecting microscope (Olympus SXZ12, Tokyo, Japan) at magnification 60× with an Infinity 3S-1UR camera (Lumenera, Ottawa, ON, Canada), allowing improved qualitative assessment of the coral damage.

Photosynthetic Activity Assessment of the Endogenous Symbionts

Corals have a mutualistic relationship with dinoflagellate algae which provide over 75% of polyps nutrition through photosynthesis (Davies 1984). Therefore, the health of these endogenous microorganisms is important to the overall health of the coral colonies. A Pulse-Amplitude-Modulation (PAM) fluorometer (Walz Heinz GmbH, Effeltrich, Germany) was used to determine the

photosynthetic activity (or yield, *Y*) of photosystem II of symbionts in the treated areas after treatments (Hagedorn et al. 2015). Specifically, we assessed the symbionts in the center and on the edge of each of the four treatment areas using the PAM. Two additional PAM values were taken at randomly chosen points of the corals in nontreated areas, providing a control. For a specific treatment, the photosynthetic yield (*Y*) of every coral fragment was averaged, and the mean and standard error of the mean (S.E.M.) were presented.

Statistical Analysis

Data analyses were performed using Prism 6.0 (GraphPad, San Diego, CA, USA) and Excel (Microsoft, Redmond, WA, USA). Percentage data were log transformed before statistical analyses. For multiple group comparisons, normality was tested graphically, and data was analyzed with ANOVA and a Tukey's posttest. All data are expressed as the mean ± S.E.M.

RESULTS

Experiment 1—S. taccada *Juice was Stable Over Time and to Heat and Frozen Storage*

Fresh extract had an SPF of 19.5 ± 0.39. Extract left for 24 h at room temperature, heated extract, and frozen extract had an average SPF of 20.2 ± 0.61, 20.5 ± 0.41, and 19.9 ± 0.30, respectively. The SPF of the extract remained stable at values in the vicinity of SPF 20 and there was no difference among the treatments ($p > .05$, $F = 1.018$) (Figure 2). The absorbance spectrum showed that the extract absorbed largely in the UV range, from 200 nm to 400 nm (Figure 3). There was a high variation in absorbance in the UVC (200–280 nm) but was relatively constant in the UVB (280–315 nm) and UVA (315–400 nm)

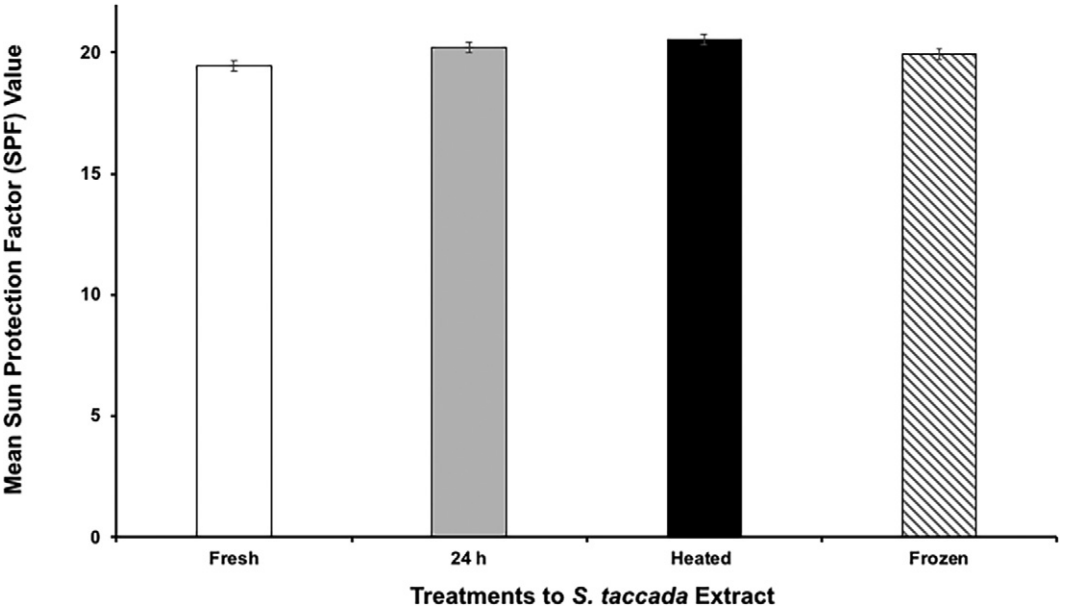

FIGURE 2. Mean sun protection factor (SPF) of *S. taccada* extract in response to different treatments. *Scaevola taccada* extract was stable to time, heat, and frozen storage and there was no difference in SPF in response to treatments ($p = .396$, $F = 1.016$, ANOVA). Initial SPF was calculated before starting experiments (Fresh): 19.5 ± 0.39. The first set of extract was left at room temperature for 24 h (24 h): SPF 20.2 ± 0.61. The second set was heated at 60 °C for 10 min and then for an hour (Heated): SPF 20.5 ± 0.41. The last set was frozen at −20 °C overnight and thawed the next morning (Frozen): SPF 19.9 ± 0.30 ($n = 10$ fruits for each suite of treatments). Bars indicate S.E.M. and small letters indicate differences among the means.

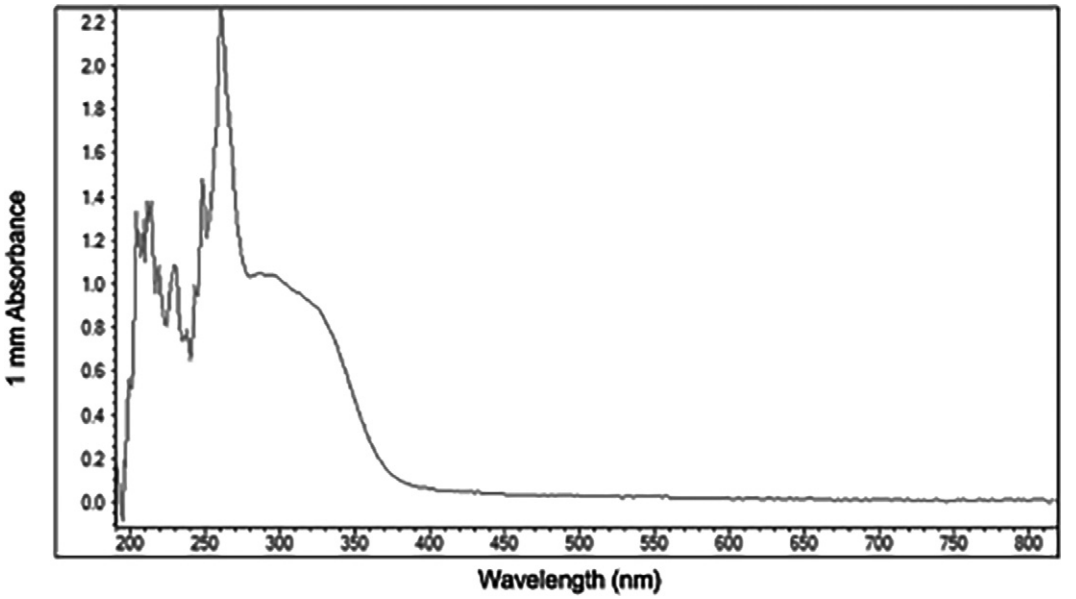

FIGURE 3. Absorbance spectrum of ripe *S. taccada* fruit extract. The highest absorbance occurred in the UVC (100–280 nm). However, most of the UVC is absorbed by the ozone layer. UVB (280–320 nm) is the most harmful to humans as it is the cause of sunburn and DNA mutation that can lead to cancers. UVA irradiance (320–400 nm) is less energetic and less harmful, but can cause skin aging.

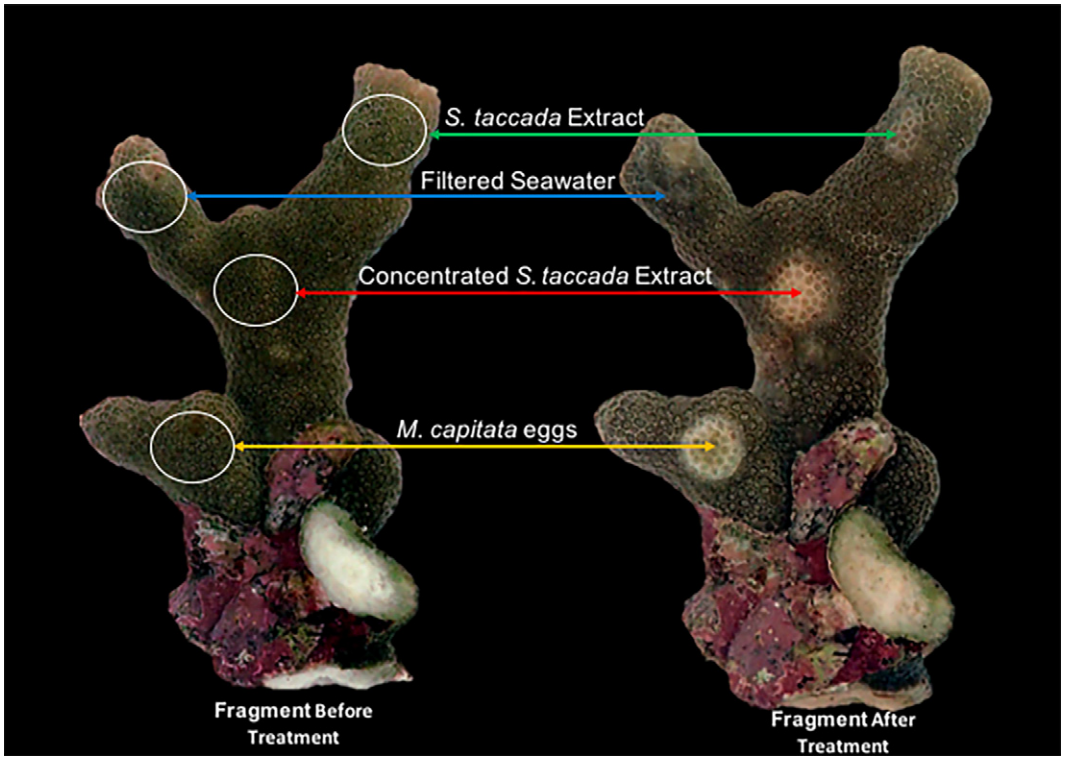

FIGURE 4. A qualitative assessment of coral damage from a macroscopic view. Colored arrows indicated the placement of the treatments (left) and response (right) of the coral. Blue: filtered seawater (FSW); green: *S. taccada* extract; red: concentrated *S. taccada* extract; orange: frozen crushed *M. capitata* eggs ($n = 7$ coral fragments for each suite of treatments).

regions with 19.6% and 24.4% absorbance, respectively.

Experiment 2—S. taccada *Juice Inhibits the Viability of Live Symbionts and Tissue in* P. compressa *Fragments*

Qualitative macroscopic observations clearly identify the areas where the treatments were applied to coral fragments (Figure 4) as all the treatments left a visible circular-shaped mark on the coral fragment. The crushed *M. capitata* egg treatment left the most prominent marks because there was generally complete erosion of the tissue within 4 days of treatment (as observed under a dissecting microscope). Concentrated *S. taccada* extract and *M. capitata* eggs produced the most prominent reactions and were different than the marks from *S. taccada* extract and the FSW control. These lighter marks could still be identified although they were less pronounced. At a microscopic scale, it was possible to observe an individual polyp's reaction to the treatments (Figure 5). Where the filtered seawater (FSW) control had been placed, polyps looked healthy and undisturbed. Under the *M. capitata* egg disks, polyps and surrounding tissues were nearly absent. Tissue erosion at the concentrated extract disk was comparable to the *M. capitata* egg treatment. There was some bleaching of the tissue surrounding the polyps from under the *S. taccada* extract disks, but the effects

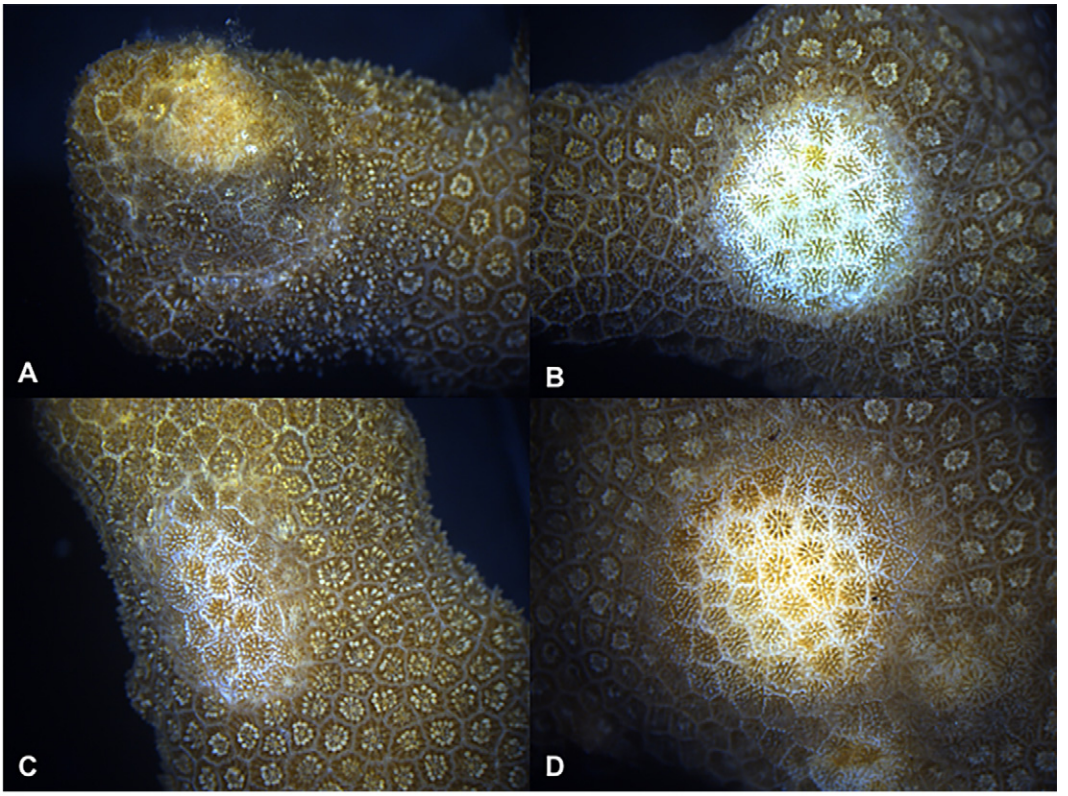

FIGURE 5. A microscopic assessment of damage to the coral tissue in response to agar-filled cap treatments. (*A*) Filtered seawater caused modest paling of the tissue under the treatment. (*B*) Frozen crushed *M. capitata* eggs were used as a negative control. The toxin in these eggs caused erosion of the tissue in the treatment area. (*C*) *S. taccada* extract resembled the FSW control in terms of damage under the treatment area. (*D*) Concentrated *S. taccada* extract appeared similar to the negative control in terms of the treatment area ($n = 7$ coral fragments for each suite of treatments).

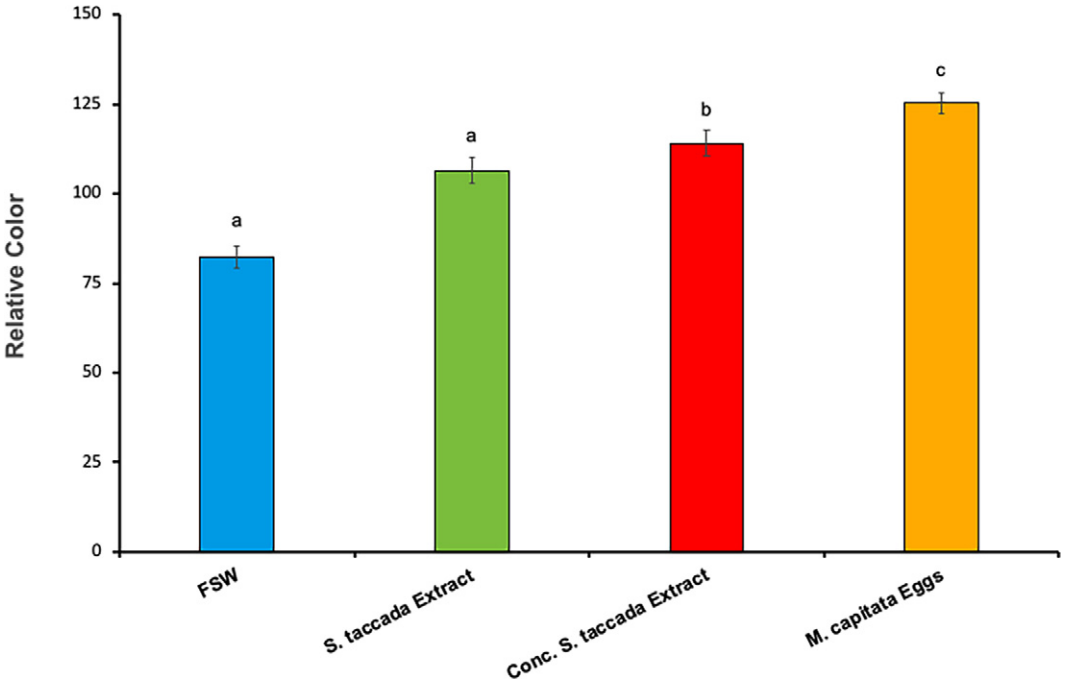

FIGURE 6. Relative color of coral tissue under the treated areas differed. When compared to the relative color of the FSW control, *S. taccada* extract (green bar) was not different; however, the concentrated *S. taccada* extract (red bar, $p = .001$) and the *M. capitata* egg toxin treatments (orange bar) were different ($p = .0001$). Bars indicate S.E.M. Different small letters indicate significant differences between control treatments (Kruskal–Wallis test statistic = 20.61, Dunn's multiple comparison test).

appeared less acute and comparable to the FSW control.

Although Figures 4 and 5 show qualitative assessments, it was possible to quantify these color changes in each treatment area (Figure 6). Crushed *M. capitata* eggs caused a $53.06\% \pm 5.05$ color reduction in comparison to the FSW control. Concentrated *S. taccada* extract caused the next greatest color reduction, $39.34\% \pm 5.74$. Lastly, *S. taccada* extract caused the smallest reduction of $29.67\% \pm 4.18$. When compared to the relative color of the FSW control, *S. taccada* extract (green bar) was not different; however, concentrated *S. taccada* extract (red bar, $p = .001$) and *M. capit*ata egg toxin treatments (orange bar) were different ($p = .0001$).

Besides loss of color, photosynthetic yield (Y) is an indication of the health of photosystem II of coral symbionts (Figure 7). By comparing the Y values of the various treatments, it was possible to determine a correlation between the color loss and the viability of the coral fragments in the treatment areas. The symbionts in contact with the FSW control had an average Y of 0.608 ± 0.01 and those in contact with *S. taccada* extract averaged 0.429 ± 0.05. Both these values indicate live symbionts in these areas. Symbionts in contact with concentrated *S. taccada* extract had an average of 0.068 ± 0.03 and those exposed to crushed *M. capitata* eggs averaged 0.051 ± 0.02. Both these values indicate nonviable symbionts in these areas. Meanwhile, the symbionts in the nontreated areas maintained robust average photosynthetic yield of 0.603 ± 0.01.

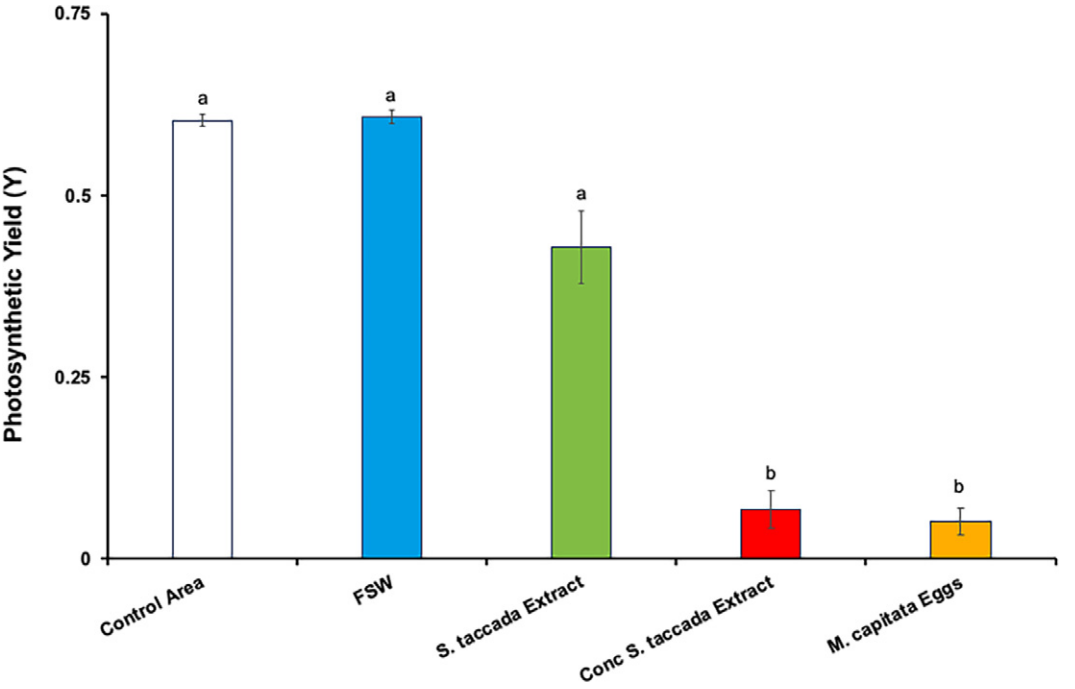

FIGURE 7. Average photosynthetic yield in coral symbionts posttreatment. Average photosynthetic yield of endogenous symbionts in the treated areas of the coral fragments. Bars (S.E.M.) with different letters are different at $p < .0001$. Only the concentrated *S. taccada* extract and *M. capitata* eggs were different than the FSW and the *S. taccada* extract. Based on their photosynthetic yield, the symbionts were dead in these affected regions, whereas in the other treatments, they were still photosynthetically active ($n = 7$ individuals, Kruskal–Wallis test statistic = 22.35, Dunn's multiple comparison test).

DISCUSSION

One of our goals was to find and examine the UV-protective properties derived from a native Hawaiian plant *S. taccada* as mentioned in Hawaiian oral traditional. The UV-blocking properties evident in *S. taccada* found in this work supported this historic oral tradition. Specifically, *S. taccada* fruit extract showed a surprising stability of the UV-absorbing property over time. Moreover, it appeared stable to freezing and heating, suggesting that this component may not be of cellular origin or a protein, respectively. Therefore, frozen storage will allow greater flexibility to manipulate the extract in view of designing future experiments without losing its protective properties. *Scaevola taccada* is not the only plant that has UV-absorbing properties; however, at an SPF 20, it is nearly twice as high as other tested plant-based compounds. Previously, the highest SPF tested by Gupta (2013) was 8.184 in *Mentha piperita* leaves.

There are two types of sunscreens that can protect against damaging UV radiation—those that physically block radiation, such as titanium dioxide, and chemical blockers that absorb harmful radiation, such as avobenzone, padimate O, octyl methoxycinnamate, octisalate, and octocrylene among others (Gasparro et al. 1998, Gupta 2013). Surprisingly, most commercial sunscreens block UVB rays effectively but offer only partial protection against UVA, even though they may be labeled as broad-spectrum products (Wang et al. 2008). The absorbance spectrum of the *S. taccada* extract showed a clear maximum in the UV range that includes both UVA and UVB, thus potentially offering a safer plant-based alternative to commercial sunscreens that can be harmful to human health (DiNardo and Downs 2018).

It is unclear why a plant produces UV-absorbing chemicals. One hypothesis would be as a protection agent against high amounts of UV radiation received due to its exposed coastal location in tropical areas of the Indian and Pacific basin (Howarth et al. 2003). These compounds may also give the plant a physiological and/or ecological advantage over other seaside plants. We noted some variation in total SPF values detected based on the plant's location. Additional collections (aside from those measured in these experiments) were made on O'ahu, specifically along Ala Moana Boulevard and on Kapi'olani Community College campus. At these locations, the fruits expressed an average SPF of 15 and 10, respectively. The causes of these variations are still unknown but perhaps soil composition, genetic variations, or other environmental conditions may play a role.

A secondary goal of this work was to determine whether the extract from *S. taccada* would harm corals. It appeared that *S. taccada* extract has some adverse effects on the health and metabolic activity of coral colonies, especially in a concentrated form. Applying the treatments directly to the coral tissues produced a reaction with the coral, including tissue loss and bleaching. Concentration played a role on how much the extract interfered with metabolic activity in corals. However, it is not clear whether it is the UV-absorbing compound or other components of the extract or a combination of these factors that caused the tissue loss and bleaching. To answer this question, methods such as hydrophilic interaction liquid chromatography (HILIC) and mass spectrophotometry may be needed to identify organic molecules in the extract and separate them into different phases of a solution (Williams et al. 2020). These components of *S. taccada* extract would need to be tested for their UV-absorbing properties.

There are a number of studies examining oxybenzone as an environmental pollutant. Lab experiments showed a clear correlation between oxybenzone presence in seawater and coral mortality, specifically disrupting larval growth and settlement (DiNardo and Downs 2018). However, it is apparent that the concentration of oxybenzone in these experiments exceeded those found in nature. Moreover, it is very difficult to accurately determine the toxicity of oxybenzone in ocean water because most seawater systems are so well mixed. Although studies in coastal waters have shown the presence of oxybenzone, it may be a relatively minor threat to coral reef ecosystems compared to the consequences of global climate change. Hawai'i is a popular vacation destination, which facilitates contact between oxybenzone from the numerous visitors and coral reef ecosystems. According to data from Dr. K. Rodgers of the University of Hawai'i's Coral Reef Ecology Lab (pers. comm), popular bathing spots like Hanauma Bay attract many swimmers each year, exposing the reefs to higher concentration of oxybenzone compared to other beaches on O'ahu.

In 2014, average global ocean temperature reached its warmest temperature since 2003 (NOAA) putting great strain on marine ecosystems, and Hawai'i experienced extensive coral bleaching events. The state of Hawai'i reported a 30% coral mortality on average related to increase in ocean temperature (pers. comm. Dr. K Rogers). On O'ahu specifically, Hanauma Bay experienced 10% mortality while West O'ahu, a less tourist-intensive area, experienced up to 50% mortality. Mortality of coral reefs correlated with a sudden increase in ocean temperature suggests that the impact of oxybenzone on coral reefs may be minor compared to rising ocean temperatures.

Similarly, our experiments involving *S. taccada* extract used a higher concentration of extract than what might be predicted to be found in nature if swimmers were to use it as a sunscreen. When corals were exposed to *S. taccada* fruit extract, they responded in a dose-dependent way with negative impacts to their epidermis and ability to photosynthesize. However, due to the large volume of water in the ocean, *S. taccada* might be diluted enough to be considered a minor threat to coral reefs. Another concern regarding oxybenzone is its persistence as an endocrine disruptor in the human body (DiNardo and Downs 2018). Research has found that 97% of urine samples contained traces of oxybenzone in the United States (Calafat et al. 2008). Thus, the

development of a healthier sunscreen is a relevant question to prevent further human health hazards and potentially a reduced hazard to the reef. By fractionating the extract to isolate the UV-absorbing component, we may be able to state more clearly in the future if the UV-absorbing component has the potential to lead to discovery of a sunscreen derived from a local plant that might be safer for coral reefs and humans alike.

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