

Sun Protection Factor Assessment of Naupaka Kahakai Extract (*Scaevola taccada*) on *Saccharomyces cerevisiae*

Keanu Rochette Yu-Tsuen

Mike Ross, M.Sc., John Berestecky, Ph.D.
Kapi'olani Community College, Honolulu, HI



Introduction

Scaevola taccada, or Naupaka Kahakai (Figure 1), is a coastal shrub indigenous to the Hawaiian Islands [1]. Indigenous knowledge suggest that the fruits of *S. taccada* have UV absorbing properties. Studies have shown that sunscreens can negatively affect coral growth and recruitment [7] and Naupaka Kahakai may represent a local alternative. *Saccharomyces cerevisiae* cultures share similar characteristics with human cells that allow us to visualize the effects of UV damage *in vitro*. Our hypothesis is that the juice of the *S. taccada* fruits will absorb UV radiation and prevent *S. cerevisiae* from cellular damages associated with UV rays.



Figure 1: (A) *Scaevola taccada* (Naupaka Kahakai) on the KCC campus, Honolulu, HI.

(B) Different stages of maturation of the fruits (Top to Bottom: Ripe, Semi-Ripe, Unripe).

Materials & Methods

- Extraction:** Juice extracted from the fruits through a sterile muslin cloth
- Spectrophotometry:** Absorbance assessment of the extract in UV range (200 nm to 400 nm)
- Compound dilution:** Dilution of extract using PBS solution to 1/2, 1/100 and 1/100.
- Stability over time:** Absorbance tested after 1h, 2h and 24h after extraction.
- Stability to Heat:** Extract heated at 60° C from 10 min and 1h.
- Stability of Frozen Extract:** Extract frozen overnight at -20° C and thawed the next morning.
- Yeast culture and UV exposure:** Inoculation of *S. cerevisiae* culture onto PDA medium. Exposure to UV radiation (253 nm) for 0 min, 0.5 min, 2 min and 5 min.

Results: UV Absorption and Stability of the Extract

The absorption spectrum shows a very strong absorption in the UVB that continues in the UVA and drops as we reach the visible light. The UVB are the cause of sunburns and alter the DNA structure causing pyrimidine dimers. If those mutations are left uncorrected by the body, they can potentially evolve in skin cancer [5,6]. From the absorption spectrum, an SPF has been calculated using the formula in Figure 2 [2]. Naupaka extract demonstrates surprising stability over time, to heat and to frozen storage with a consistent SPF value of 20 (Figure 3). This allows for more flexibility as we design future experiments using the extract.

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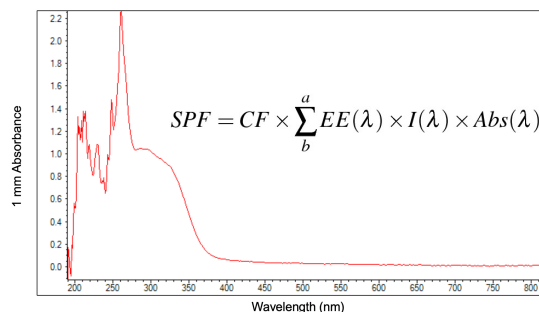


Figure 2: Absorption spectrum for *S. taccada* ripe fruit juice extract. SPF Formula with a = 290 nm and b = 320 nm.

Stability of the Sun Protection Factor (SPF) Provided by Naupaka Extract to Different Treatments

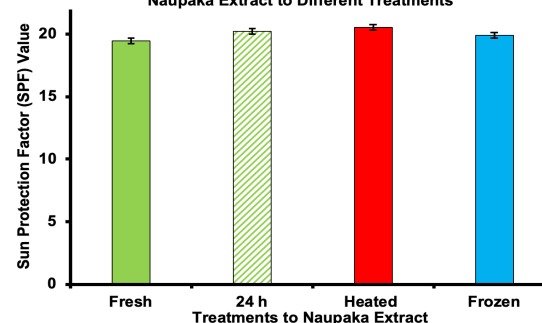


Figure 3: Stability of Naupaka Extract over Time, to Heat Treatment and Frozen Storage.

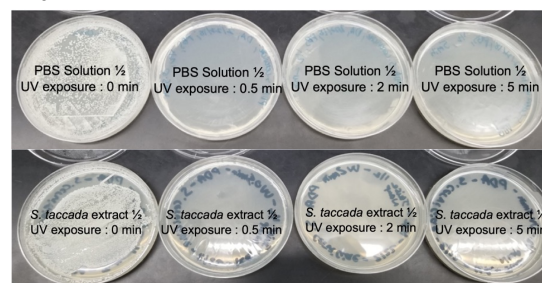


Figure 4: Control plates with PBS solution at 1/2 dilution (0 min, 0.5 min, 2 min and 5 min) Suspensions at 1/2 dilution with *S. taccada* juice (0 min, 0.5 min, 2 min, 5 min)

Results: Viability of Yeast Cultures

In the second experiment, we tested the effects of Naupaka extract on yeast cultures. The results tell us that the *S. taccada* juice does not inhibit the growth of *S. cerevisiae* when added to the suspensions. The control plates showed that the cultures exposed to UV rays had no viability while the unexposed plates presented uninhibited growth. Similarly, plates containing the *S. taccada* extract showed no viability when exposed to UV radiation of 253 nm and uninhibited growth when unexposed.

Conclusion

- The absorption spectrum from the Nanodrop™ spectrophotometer indicated that the Naupaka (*S. taccada*) juice does contain UV absorbing properties that can be exploited, with a calculated SPF of 20.
- It appears that the SPF of the extract is stable to time, to heat and to frozen storage.
- The plates exposed to UV radiation (253 nm) showed no signs of viability with the conditions given by the experiments. Therefore, the results do not support the original hypothesis.
- These results suggest that we need to critically review our protocols and methods before we repeat the experiment.

Ongoing Research

Naupaka extract was heated at 60° C and concentrated to 4 times its original concentration. Fresh and concentrated Naupaka extract were applied on coral colonies (*Porites compressa*). A positive control of filtered sea water and a negative control of montiporic acid were used to assess coral damages. Naupaka extract caused significant coral damages and severity of the damages or proportional to the concentration of the extract. The extract was assessed with a pH value of 5. A titration of the extract will be performed to neutralize its pH and repeat the coral experiments.

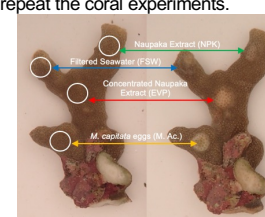


Figure 5: Macroscopic assessment of coral damages.

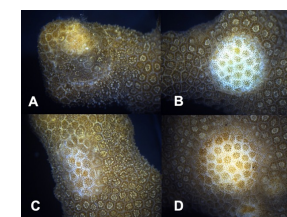


Figure 6: Coral damages from filtered sea water (A), montiporic acid (B), fresh Naupaka (C) and concentrated Naupaka (D).

- Howarth, D. G., Gustafsson M. H. G., Baum D.A., and Motley T. J. 2003. Phylogenetics of the genus *Scaevola* (Goodeniaceae): Implication for dispersal patterns across the Pacific Basin and colonization of the Hawaiian Islands. *American J. Bot.* 90: 915-923.
- Gupta, D. (2013). UV absorbing properties of some plant derived extracts. *Res. J. Chem. Environ. Sci.* 1, 34-36.
- Fonseca, A. P., & Rastel, N. (2013). Determination of sun protection factor by UV-vis spectrophotometry. *Health Care*, 1(1), 4.
- Ray, A., Gupta, S. D., & Ghosh, S. (2013). Evaluation of anti-oxidative activity and UV absorption potential of the extracts of *Aloe vera* L. gel from different growth periods of plants. *Industrial Crops and Products*, 49, 712-719.
- Kielbassa, C., Roza, L., & Epe, B. (1997). Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis*, 18(4), 811-816.
- DiNardo, J. C., & Downs, C. A. (2018). Dermatological and environmental toxicological impact of the sunscreen ingredient oxybenzone/benzophenone-3. *Journal of cosmetic dermatology*, 17(1), 15-19.
- Lee, B. K., Corvalan, N., & Sarum, J. Z. (2018). The Controversy of Sunscreen Ingredients: Examining the Relationship Between Oxybenzone and Butylparaben on Stylophorum Piliella. *Exigence*, 2(1), 1-23.
- Ramos, S., Homem, V., Alves, A., & Santos, L. (2015). Advances in analytical methods and occurrence of organic UV-filters in the environment—a review. *Science of the total Environment*, 526, 278-311.
- Hagedorn, M. A., Farrell, V., Carter, N., Zuchowicz, E., Johnston, J., Padilla-Gamino, S., Gunasekera and V. Paul. 2015. Effects of toxic compounds in *Montipora capitata* on exogenous and endogenous symbionts performance and fertilization success. *PLoS One*, 10(2): e0118364.