Cooperative Extension
University of Hawai'i at Mānoa
College of Tropical Agriculture and Human Resources

Mamaki
Hawai`i's Endemic Tea
(Pipturus albidus)

J. Sugano, R. Saito, J. Uyeda, S. Fukuda, S. Migita, R. Corrales , T. Radovich, and J. Silva

University of Hawai`i at Mānoa
College of Tropical Agriculture and Human Resources
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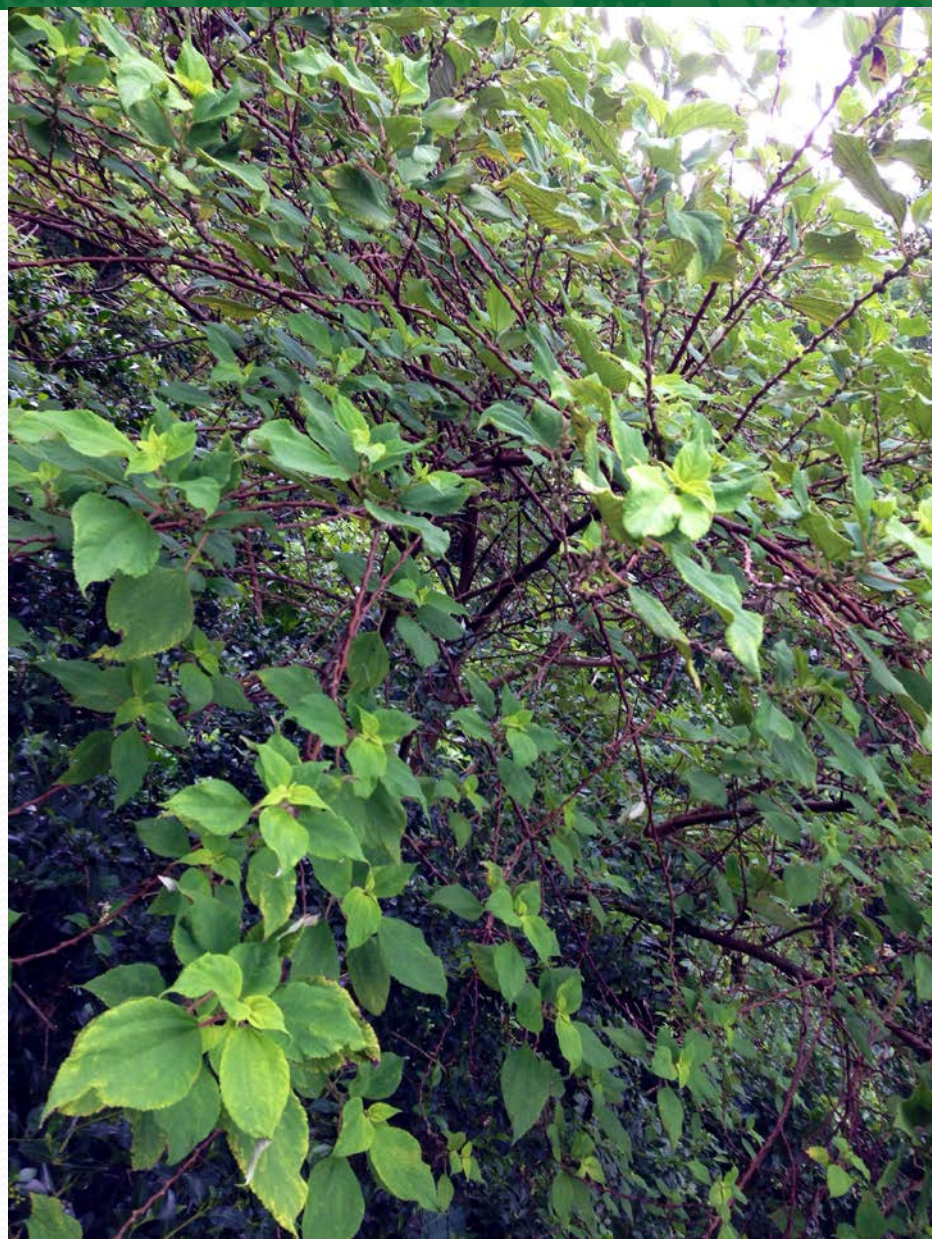
Mamaki

- Hawaiian endemic plant (Wagner, 1990)
- Hawaiian nettle without thorns
- Location: Typically found in the understory of the wet forest, on all islands except for Kaho'olawe and Ni'ihau.



Source: http://www.hawaiiannativeplants.com/ourplants/mamaki/
http://www2.hawaii.edu/~eherring/hawnprop/pip-albi.htm

Endemic
Found only in Hawaii

Mamaki Uses

- Dye for clothes
- Food for insects
- Medicine (tea/ tonic, or aspirin)

Source: http://www.hawaiiannativeplants.com/ourplants/mamaki/
http://www2.hawaii.edu/~eherring/hawnprop/pip-albi.htm

DYE

https://i.pinimg.com/564x/00/95/85/009585a17b5f42cdf68f5d1530d196b0.jpg
https://commons.wikimedia.org/wiki/File:Kapa_or_Tapa_cloth,_Hawaii,_possibly_collected_by_Captain_James_Cook,_before_1869_-_Pacific_collection_-_Peabody_Museum,_Harvard_University_-_DSC05750.JPG

Food for the Kamehameha Butterfly (endemic)

Photo by Will Haines. Courtesy UH.

Kamehameha Butterfly Project

Kent Kobayashi & TPSS students


MEDICINE

Mamaki: Research Foundation

- Mamaki tea has significant levels of total & specific antioxidants
- UH CTAHR Research (Kartika et al. (2007) shows, three major polyphenols (phyto-chemicals) in mamaki leaves are [1]:
  - Catechins are a type of antioxidant commonly found in red wine, chocolate, berries, and apples
  - Chlorogenic acid is an antioxidant. commonly found in root vegetables such as carrots, radishes, turnips, and burdock
  - Rutin is a flavonoid (plant compound) in the polyphenol family. Rutin is commonly found in red wine, buckwheat, citrus, and tomato skin

Plant Compounds in Mamaki

- The concentrations of catechins and rutin in Mamaki leaves are higher than commercial tea leaves [2,3]:
  - Gyokuro green tea leaves
  - Chinese oolong tea leaves, and
  - Kenya black tea leaves
- Purple leaf cultivars of mamaki had higher catechins (Karita et al.) than other mamaki varieties , however, total antioxidants vary over seasons and between cultivar selections [1].

Major Phenolic Acids and Total Antioxidant Activity in Mamaki Leaves, *Pipturus albidus*

H. Kartika, Q.X. Li, M.M. Wall, S.T. Nakamoto, and W.T. Iwaoka

ABSTRACT: Three phenolic acids, (+)catechins, chlorogenic acid, and rutin, were identified and quantified in Mamaki leaves using a liquid chromatograph-mass spectrometer technique. Concentrations of (+)catechins, chlorogenic acid, and rutin varied from 1.1 to 5.0 mg/g of Mamaki leaves as determined in the extract using 0.5% acetic acid in 90% aqueous methanol. This study also quantified total antioxidant capacity using the photochemiluminescence method, which was expressed in equivalents to ascorbic acid (AA). Mamaki teas brewed for 30 min contained total antioxidant activity (TAA) between 238 and 259 mg AA/g of tea. Mamaki teas brewed for 1 h and stored at 4 h, 1 d, and 3 d at 4 °C had available TAA 293, 271, 172, and 163 mg AA/g of tea leaves, respectively. The concentrations of (+)catechins and rutin in Mamaki leaves are compared to other types of popular teas. Mamaki teas contained relatively low amounts of TAA compared to green teas and Lipton teas.

Keywords: antioxidant, herbal drink, Mamaki tea, *Pipturus albidus*, polyphenols

Introduction

Phenolic compounds are commonly found in all plants as secondary metabolites. They are derived from phenylalanine and tyrosine in the shikimic acid pathway (Hermann 1995). They are produced in response to environmental stress such as microbial infections, UV radiation, and chemical stressors, rather than involvement in plant growth and development (Dixon and Paiva 1995). Polyphenols in plants include simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins (Dixon and Paiva 1995). These phenolic compounds contribute to color, bitter and astringent taste, flavor, odor, and antioxidant properties.

Recently, there has been a growing interest in the study of nutraceuticals in plants due to their antioxidative, mild estrogenic, and hypolipidemic activity. Research indicates that phytophenolics are potent antioxidants in scavenging free radicals and inhibiting lipid peroxidation in human tissues (Rice-Evans and others 1997; Marja and others 1999; Sugihara and others 1999). They play roles as reducing agents, metal chelators, singlet oxygen quenchers, and hydrogen donors (Rice-Evans and others 1997; Kuo and others 1998; Yoshino & Murakami 1998; Marja and others 1999; Sugihara and others 1999). Furthermore, studies show that these phenolic compounds are linked with lower occurrence of and lower mortality rates from various human diseases (Block and others 1992; Ames and others 1993; Geleinjese and others 2002).

Traditionally, Mamaki plants (*Pipturus albidus)* were used by native Hawaiians to ease childbirth, to discharge blood, and to alleviate listlessness (Chun 1994). Previous studies found that parts of Mamaki plants have antimicrobial, antiviral, and antifungal properties (Locker and others 1995). Recently, Mamaki tea leaves became well known in therapeutic usage for alleviating various preexisting diseases (Chun 1994). According to local folklore, Mamaki leaves are a potential natural therapeutic medicine in regulating blood sugar levels, blood pressure, and cholesterol levels (Chun 1994).

However, due to limited information available on the Mamaki plant and especially the leaves, research on its chemical and antioxidant properties is necessary. Mamaki leaves are commonly prepared as herbal teas, and consumed as fresh-brewed teas or as cold herbal teas. Thus, the purpose of this study was to identify and quantify major polyphenols present in Mamaki leaves and quantify the total antioxidant capacity in Mamaki teas after brewing, steeping, and storing them for 3 d at 4 °C.

Materials and Methods

Identification and quantification of Mamaki leaves

Chemicals. Optima grade solvents including methanol, acetic acid, and formic acid were obtained from Fisher Scientific (Pittsburg, Pa., U.S.A.). (+)Catechins, chlorogenic acid, caffeine, and rutin were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Water was purified and filtered at all times. The water was passed through a Milli-Q water purification system (Millipore Corp., Bedford, Mass., U.S.A.) set at 18 MΩ-cm resistance. HPLC solvents were filtered through Nylon 66, 47-mm i.d., 0.45-μm pore size (Millipore Corp.).

(+)Catechins, chlorogenic acid, and rutin were each dissolved in methanol and sonicated until the powder dissolved into solutions. (+)Catechins were prepared at 200, 400, and 800 μg/mL. Concentrations of chlorogenic acid were 100, 500, and 1000 μg/mL. Rutin was prepared with concentrations of 80, 400, and 800 μg/mL.

Mamaki sample preparation. Four varieties of fresh Mamaki leaves were hand harvested, placed in labeled zip lock bags and immediately chilled in a cooler. They were distinguished by leaf physical characteristics as (1) purple veined and purple leaves, (2) green veined and green leaves, (3) a hybrid plant with green leaves and purple veins, and (4) the so-called "panaewa" by the farmer with green leaves and light pink veins (Figure 1). For each variety, Mamaki leaves were ground in a clean vegetable chopper with dry ice until it gave a uniform powder. Ground Mamaki leaves were then lyophilized at 0.2 Pa for more than 48 h and stored in an airtight container in the refrigerator after processing.

Extraction of polyphenols. Mamaki samples (0.5 g) were extracted with 12 mL of 90% aqueous methanol containing 0.5% acetic acid. The solution was sonicated for 3 min followed by centrifugation at 3000 rpm for 15 min. After 3 times of extraction, the supernatants were dried with a speed-vacuum centrifuge (Vacufuge™, Eppendorf, Germany). The residues were dissolved in methanol and filtered through a PTFE 0.25-μm membrane filter (Millipore Co.). The solution was concentrated to a final volume of 1.5 mL under a gentle nitrogen gas flow.

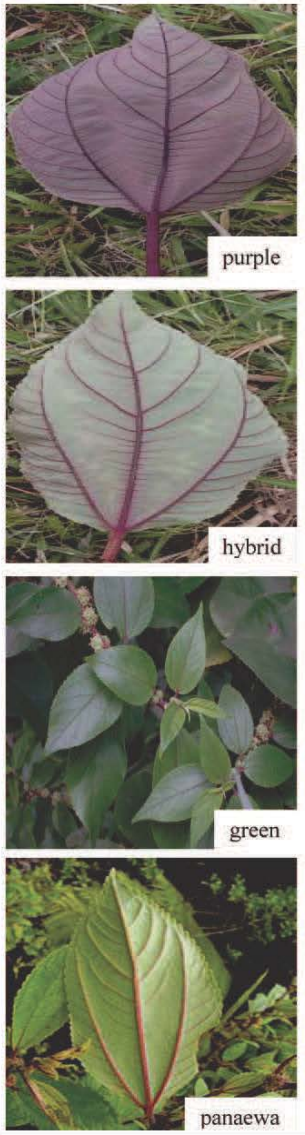


Figure 1 — Four different varieties of Mamaki leaves (*Pipturus albudus*).

LCMS conditions. The LC–MS system consisted of an Agilent 1100 series liquid chromatograph, a photodiode array (PDA) detector, a single quadruple mass spectrometer, a vacuum degasser, a binary pump, an autosampler, and an electrospray ionization (ESI) source (Agilent Technologies, Wilmington, Del., U.S.A.). Mass spectra were acquired in negative ion mode in a mass range of m/z 110 to 1000. The compounds were monitored at 250, 280, 320, 370, and 510 nm. The drying gas was nitrogen at a flow rate of 10 L/min, creating a nebulizing pressure of 25 psi. The fragmentation voltage was 120 V. The capillary voltage was 4 kV.

LC separations were performed with a Phenomenex Luna, C-18 column (4.6 × 250 mm, 5-μm i.d.) joined with a guard column (4 × 3 mm i.d.) at 35 °C. The gradient elution was set up according to the method of Sakakibara and others (2003) with some modification. Gradient elution was carried out with solvent A (0.1% formic acid solution) and solvent B (100% methanol), delivered at a constant rate of 1 mL/min as follows: initially 100% of solution A; for the next 15 min, 70% of solution A; for another 30 min, 65% A; for another 20 min, 60% A; for another 5 min, 50% A; and finally for the last 25 min, 0% A. The injection volume was 10 μL.

Statistical analysis

Concentrations of catechins and rutin in the Mamaki tea leaf samples were compared with other commercial teas using a 1-tailed probability of a Z-test. The Z-test is calculated as $1 - [(x_{bar} - \mu_0)/(\sigma/\sqrt{n})]$. A 1-tailed probability Z-test was selected to see if the average of the Mamaki samples is greater than other teas.

The total antioxidant activities (TAA) in the Mamaki tea were also compared to other published data using the 1-tailed probability Z-test.

Antioxidant activity of Mamaki tea

Mamaki tea preparation. Three varieties of dried Mamaki leaves, (1) purple veined and purple leaves, (2) green veined and green leaves, and (3) a hybrid plant with green leaves and purple veins, were analyzed for the antioxidant analysis (Figure 1). For tea preparation of each variety, a water extract was made by adding 100 mL of boiling water to 1 g of dried leaves and allowing the tea to stand for 30 min in a covered container. The Mamaki teas were prepared in triplicates and, after steeping, analyzed for their water-soluble antioxidant activities.

In the storage study, teas from the purple variety were prepared by steeping 1 g of leaves in 100 mL of boiling water followed by steeping for 60 min. The Mamaki teas were then stored for 4 h, 1 d, and 3 d at 4 °C. The Mamaki teas were prepared in triplicates and each sample was analyzed 3 times to ensure accuracy of measurement.

Antioxidant study. The antioxidant activities were measured with a Photochem® system (Analytik Jena AG, The Woodlands, Tex., U.S.A.). The system enables the quantification of antioxidant capacity of water-soluble substances based on photochemiluminescence (PCL). This includes photochemical excitation to generate free radicals (superoxide anion radicals) followed by luminescence detection (Popov and Lewin 1994). The free radicals generated by the optical excitation of the photosensitizer substance are partly eliminated by the reaction of antioxidants in the sample to be analyzed. In a measurement cell, the luminescence of the detection substance

MS 20070357 Submitted 5/11/2007, Accepted 8/5/2007. Authors Kartika, Nakamoto, and Iwaoka are with Dept. of Human Nutrition, Food, and Animal Science, and author Li is with Molecular Biosciences and Bioengineering, Univ. of Hawaii at Manoa, 1955 East West Road, Honolulu, HI 96822, U.S.A. Author Wall is with U.S. Pacific Basin Agriculture Research Center, USDA, ARS, P.O. Box 4459, Hilo, HI 96720, U.S.A. Direct inquiries to author Iwaoka (E-mail: iwaoka@hawaii.edu).

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Expand the Mamaki Tea Market in Hawaii

- Sought mamaki varieties from neighbor islands
- Grew out plants from seeds
- Installed an observational trial at the UH Research Station
- Selected lines with commercial promise


Strain Hunting
Forest & Kim Starr, Maui

Native Nursery, Maui


Pat Conant & Randy Hamasaki, Hawaii


Seeds: Genetically Different


In production in 6 months or less


ELMER'S
UPS CLASSES
INTNL AIR LTR
USPS CLASSES
SPECIAL SERVICES
ON/OFF
ZERO
1ST PRIOR
PARCEL
EXPRESS
INT AIR LTR
1ST PREST
CERTIFIED
REGISTERED
RET RCPT
INSURANCE
COD
GROUND
3 DAY SEL
2ND DAY
METER
ENTER

Selection: Weight or Antioxidants?

Identify high yielding varieties with superior taste

- Our field observations include:
  - Mamaki does better under shade than in full sun
  - Higher yielding plants do not have dark color veins
    - Should we go for yield or antioxidants?
  - Increased color in leaves results in bitter tea
    - Increased antioxidants affect taste?

Asian Teas (Green, Black, Oolong, and White)

Photo source: http://www.naturallivingideas.com/10-amazing-benefits-of-matcha-green-tea/

Photo source: Various online resources


Starbucks is committed to reducing our environmental impact through increased use of post-consumer recycled materials. Help us help the planet.
First-ever 10% post-consumer fiber cup
60% post-consumer fiber sleeve
Intended for single use only.
© 2005 Starbucks Coffee Company. All rights reserved.
U.S. Patent no. 5,205,473 and no. 6,863,644 and related foreign patents pending.
www.starbucks.com/wayiseeit
CAUTION: VERY HOT
Decaf
Milk
Shots
Custom
Syrup
Drink


Shade effect


Antioxidants or Taste?


Improve Taste via Processing


Old Processing Method: Japanese Tea Method


Consulted Commercial Mamaki Tea Growers


Wash

Dehydrate using a commercial dryer

Rolling boil system vs hot water steeping


MAMAKI LEAF PROJECT
UNIVERSITY OF HAWAI'I AT MĀNOA


HAWAII

Taste Testing

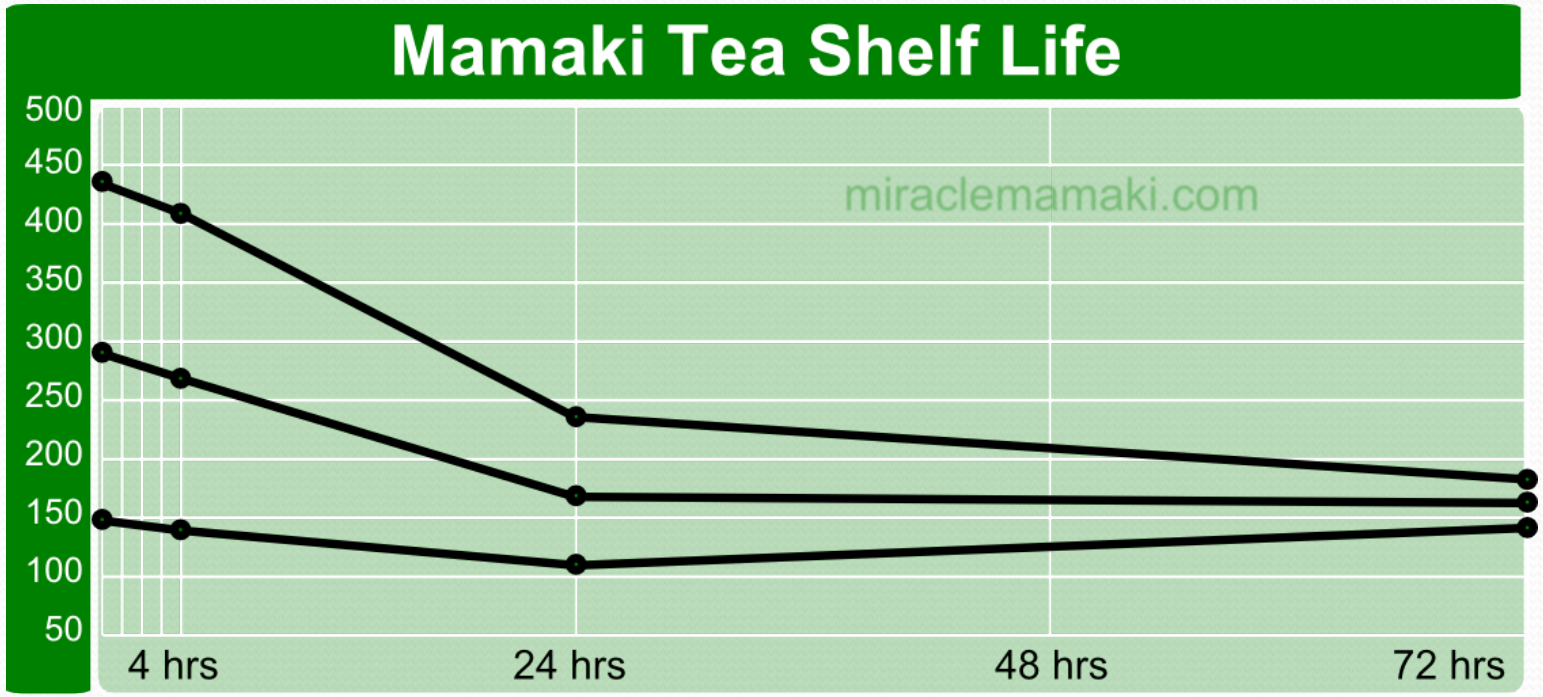
Mamaki Tea Shelf Life

Total antioxidant activity in PURPLE Mamaki teas after brewed for 1 hour and stored at 4° C (mg AA/g tea)*

Freshly Brewed	293 ± 145
4 hours later	271 ± 134
24 hours later	172 ± 65
72 hours later	163 ± 18

* Values are means of triplicate samples & 9 analyses.

miraclemamaki.com



RESOURES CITED:

[1] Kartika, H; Li, Q X; Wall, M M; Nakamoto, S T; Iwaoka, W T; Major phenolic acids and total antioxidant activity in Mamaki leaves, Pipturus albidus. Journal of Food Science—Vol. 72, Nr. 9, 2007

[2] Du Toit R, Volsteedt Y, Apostolides Z. 2001. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. Toxicology 166:63–9.

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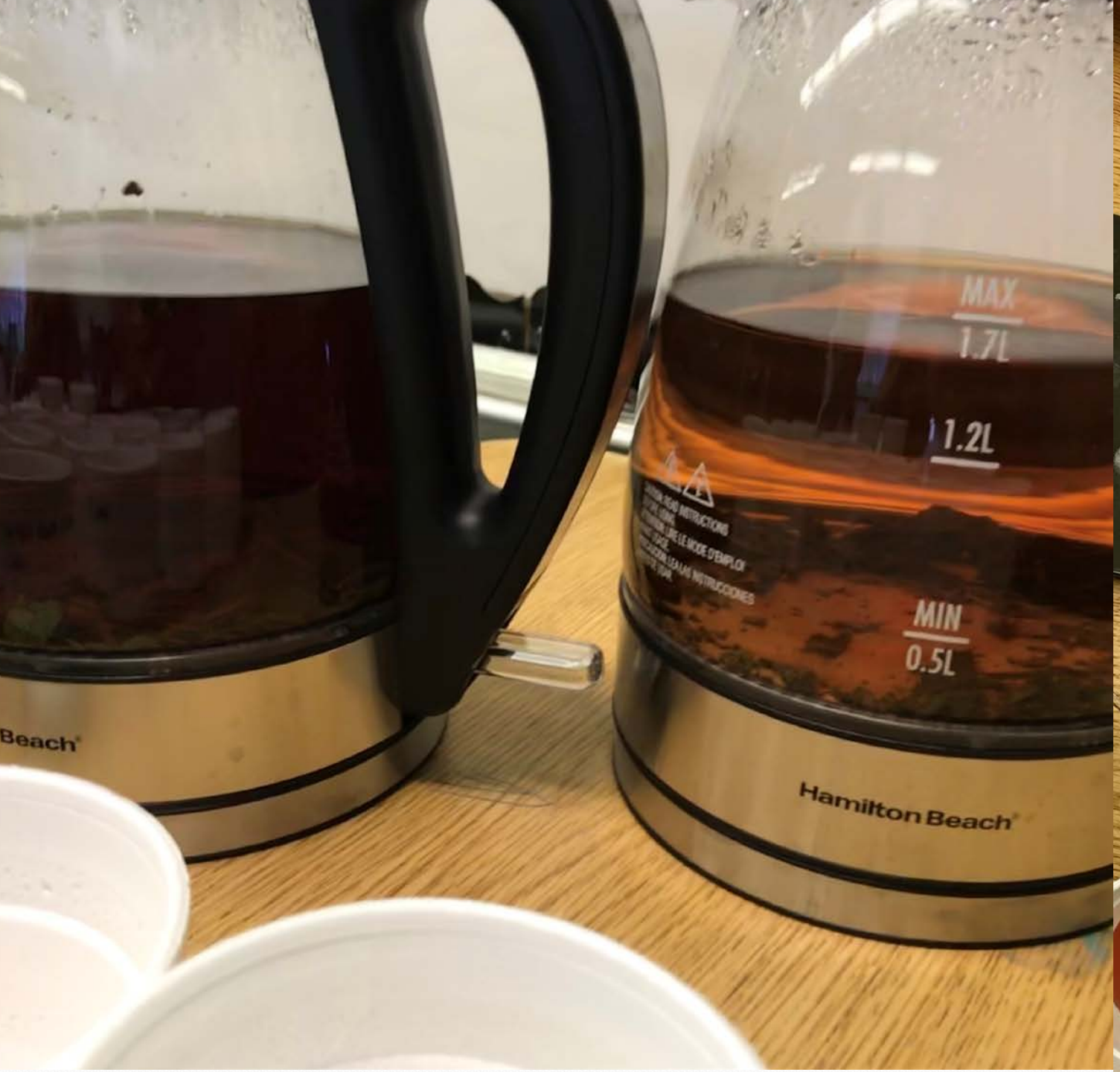
School Experiments

- Fresh or Dried
- Hot or Cold

https://studiamirabilium.com/2011/08/25/small-victories/


MAX
1.7L
1.2L
MIN
0.5L
Hamilton Beach

For More Information

Jari S.K. Sugano
University of Hawaii at Manoa
College of Tropical Agriculture and Human Resources
Department of Plant and Environmental Protection Sciences
Wahiawa Extension Office
suganoj@ctahr.hawaii.edu
622-4185