Investigating volatiles as the secondary metabolome of *Piper methysticum* from root powder and water extracts using comprehensive two-dimensional gas chromatography

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Abstract

Ethnopharmacological relevance

Kava (*Piper methysticum* G. Forst) is a plant grown in the Pacific that is used in traditional medicines. The roots are macerated and powdered for consumption as a beverage in social settings as well as in ceremonies. Other types of preparations can also be used as traditional medicines. There has been an increase in demand for kava as there is continued traditional use and as it is becoming utilized more both socially and medicinally outside of Oceania. Currently, most research of this plant has focused on bioactive kavalactones and flavokawains, and there are few studies focusing on the other compounds that kava contains, such as volatile and semivolatile components.

Aim of the study

This study investigated the kava volatile organic compound (VOC) profile from nine different commercially available samples of dried, powdered kava root sourced across the Pacific region.

Materials and methods

The headspace above the kava samples was analyzed, both from the root powder as originally purchased and by performing a scaled-down extraction into water mimicking traditional preparation of the beverage. The headspace of each sample was extracted using solid-phase microextraction arrow (SPME Arrow), followed by analysis using comprehensive two-dimensional gas chromatography – quadrupole mass spectrometry/flame ionization detection (GC×GC-qMS/FID). The superior peak capacity of GC×GC was invaluable in effectively separating the complex mixture of compounds found in all samples, which enabled improved monitoring of minor differences between batches.

Results

Dry root powder samples contained high levels of β-caryophyllene while water extracted samples showed high levels of camphene. Many alcohols, aldehydes, ketones, terpenes, terpenoids, and aromatics were also characterized from both types of samples. All water extracted samples from the different brands followed similar trends in terms of compounds being detected or not. Additional major compounds found in water extracts included benzaldehyde, hexanal, methoxyphenyloxime, camphor, limonene, 1-hexanol, endoborneol, and copaene. While some samples could be differentiated based on brand, samples did not group by purported geographic origin.

Conclusions

This study provides foundational data about a different subset of compounds within kava than previous research has studied, and also informs the community of the compounds that transfer into the consumed beverage during the traditional means of preparing kava.

Keywords
Quality traditional medicines; Analgesic; Terpenes; Anxiolytic; Phytochemistry; Traditional medicine Asia & Oceania; *Piper methysticum*; Kava; Oceania; Gas chromatography; Multidimensional separations

Abbreviations

$^{1}t_R$: Retention time in the first dimension
$^{2}t_R$: Retention time in the second dimension
CWR: Carbon wide range
FID: Flame ionization detector
GC: Gas chromatography
GC×GC: Comprehensive two-dimensional gas chromatography
GC×GC-MS: Comprehensive two-dimensional gas chromatography coupled to mass spectrometry
GC×GC-qMS/FID: Comprehensive two-dimensional gas chromatography coupled to quadrupole mass spectrometry/flame ionization detection
MS: Mass spectrometry
PCA: Principal component analysis
PDMS: Polydimethylsiloxane
RI: Retention index
RMF: Reverse match factor
SPME: Solid-phase microextraction
VOC: Volatile organic compound

1. Introduction

1.1. Overview and background

Kava[1] (*Piper methysticum* G. Forst) is a plant endemic to the Pacific Islands. It was first domesticated in the western Pacific (most likely what is today Vanuatu) and was widely dispersed across the Pacific by indigenous migrants as part of their agricultural assemblage (Lebot et al., 1997). Its roots are macerated or powdered and used to prepare a beverage consumed in social gatherings and ceremonies. The beverage and other kava plant preparations from roots as well as from above-ground parts are used as traditional medicines. Outside Oceania, demand for kava has increased for both social and medicinal consumption (Baker, 2012; Lebot et al., 1997).

To date, much of the focus on the chemistry and pharmacology of kava has been on the bioactive kavalactones and flavokawains. They have been extensively characterized, and many aspects of their physiological effects are generally understood (Lebot et al., 1997; Shimoda et al., 2015;

[1] Kava is known by names across the Pacific (e.g., `awa in Hawai`i, sakau in Pohnpei, yaqona in Fiji). The authors use kava in this paper because this is the common name most frequently applied to it as a global commodity. A full discussion of regional linguistic variation in names for *Piper methysticum* use can be found in Lebot, Merlin, and Lindstrom (1997).

Showman et al., 2015; Singh, 2004; Xuan et al., 2008). However, kava plants contain a diverse secondary metabolome extending beyond these well characterized constituents, including many compounds capable of affecting the physiological responses of human cells and tissues (Shimoda et al., 2012). While the non-kavalactone fraction of kava has been noted and recognized in part (Jaiswal et al., 2020; Xuan et al., 2008), no studies to date have focused on comprehensively characterizing the non-kavalactone fraction and its potential for contribution to the therapeutic effects of kava. The terpenes, terpenoids, and other volatile and semi-volatile components are especially promising, since many are known to have therapeutic effects (Baron, 2018; Cox-Georgian et al., 2019; de Cássia da Silveira e Sá et al., 2017). This paper seeks to fill the gap by contributing to an improved characterization of the volatile and semi-volatile chemistry of kava as a step towards further understanding the broader potential of the kava secondary metabolome.

Second, this paper seeks to use these findings as a case study for discussing the use of such characterizations of bioactive secondary metabolomes as a means of democratizing the availability of efficacious indigenous therapeutics. Despite significant evidence indicating the complexity and sophistication of indigenous pharmacopoeia, the promise of traditional medicines as sources for safe, efficacious therapies backed by evidence largely remains out of reach (Jansen et al., 2021; World Health Organization, 2021). A typical reductionist Western pharmacological approach, focused on isolating single bioactive constituents, risks missing a significant portion of the potential of plant secondary metabolites, while also favoring the development of medicines destined to be unavailable to the majority of the world's population. This paper frames the discussion of the volatile and semi-volatile kava metabolome around these broader issues.

1.2. Broader ethnopharmacological themes and concerns

Contemporary kava use illustrates some of the consequences of a reductionist Western pharmacological approach. Much of the research to date into the pharmacology of kavahas focused on a single group of bioactive compounds (kavalactones) and on the anxiolytic effects of kava. This narrow focus means that the potential of a wider range of therapeutic uses is underappreciated. Also, indigenous knowledge and practice associated with these additional uses is at risk of being lost.

Kava has a well-documented record of historical and ongoing indigenous use for a wide range of conditions, prepared both as single ingredient medicines and in combination with other components, and administered as both internal and topical medicines (Lebot et al., 1997; Winter, 2004). Its diuretic and sedative effects are widely appreciated across the range of its indigenous use, and it is employed in ways that reflect this. However, the list of conditions for which kava preparations are used is much broader than this. These can roughly be categorized (following Lebot, Merlin, and Lindstrom (1997)) as: (1) urogenital infections (gonorrhea, urinary tract infections); (2) women's reproductive health (to induce contractions, facilitate delivery, stimulate milk production; also, for dysmenorrhea, contraception, and as an abortifacient); (3) pain and inflammation (headaches, toothaches, sore throats, body aches, rheumatism, arthritis); (4) wounds and skin diseases (usually applied externally on wounds and boils, and to treat leprosy); and (5) respiratory conditions (asthma, irritation of the respiratory tract, tuberculosis, pulmonary

pains)(Lebot et al., 1997). This list is not exhaustive, and some ethnographic reports of conditions treated are not easily reduced to broad western categories (see, for example, (Winter, 2004), for descriptions of kava being used as therapy for conditions described in terms closer to indigenous Hawaiian conceptualizations of health and illness). Also, note that, while the sedative effects of kava are recognized, and are in some cases used therapeutically to help with sleeping problems, this does not appear to be its primary ethnomedicinal use.

By contrast, nontraditional kava use (both medical and recreational) focuses on its anxiolytic properties and/or its sedative and mildly intoxicating effects. This is likewise reflected in the research into the medicinal and pharmacological potential of kava, which, relative to the full range of conditions and preparations found in ethnographic practice, represent only a select portion of this traditional scope and breadth of use. In many ways, this represents a transformation and translation of indigenous practices into other cultural ways of understanding health, illness, and intended effect (Baker, 2012). While this reduction and translation of scope is to be expected as part of the process of medicines in motion globally (Etkin, 1992; Etkin et al., 1990; Jansen et al., 2021), the concern is that much of the therapeutic potential reflected in indigenous use might be ignored.

The goal of focusing on these themes is to redress this reductionistic trend. The data about the volatile and semi-volatile constituents in kava, presented here in a degree of fidelity previously unavailable, allow for consideration of the broader ethnopharmacological understandings represented in the ethnographic record. Through the lens of a network pharmacology approach (Hopkins, 2008; Jansen et al., 2021), correlations and patterns across multiple species and groups of constituents can be appreciated. This may ultimately provide guidance for therapeutics from readily available sources for those without access to western pharmaceuticals.

This research examined the volatile and semi-volatile components within kava to provide a more detailed understanding of its chemical composition. The experiment incorporated headspace analysis from dried kava powder and water extracts from the same powder samples to examine differences between the product source and the prepared beverage typically consumed. The sampling was performed using headspace solid-phase microextraction arrow (SPME Arrow) and comprehensive two-dimensional gas chromatography - quadrupole mass spectrometry/flame ionization detection (GC×GC-qMS/FID). This technique is well suited for analysis of complex chemical mixture samples due to its high peak capacity and selectivity. Kava samples from different regions and suppliers were tested to investigate and represent a range of different samples as the core profile of chemical compounds.

2. Material and Methods

2.1. Sample preparation

This research was conducted by analyzing the dry kava powder directly and then extracting the same kava samples in water and analyzing the headspace of the extracts. The goal was to perform both treatments on the same kava sample (i.e. directly extracting from the vial in which the powder was analyzed first) to have paired data for the two treatments rather than introduce

sampling error by using fresh samples for the extracted batch. Given the gentle parameters used, the SPME Arrow sampling from the powder did not significantly alter the samples for the extract analysis. Five replicates of each sample were weighed into separate vials and analyzed. The samples were stored in a cool, dry location and were individually extracted using a microscale version of the kava preparation process (described below), and the extracts were analyzed for each vial. Sample preparation was performed by first using 1 g of dry kava powder in a 20 mL headspace vial with magnetic screw cap (Restek Corporation, Bellefonte, PA, USA). Samples were weighed on an analytical balance and masses were recorded. Nine kava root powder samples were analyzed as listed in Table 1. An empty 20 mL headspace vial (Restek Corporation) was included as an instrument blank.

2.2. Kava extraction method

To each vial containing a powdered kava sample, 10 mL of bottled drinking water (Hawaiian Isles, Honolulu, HI, USA) was added with a micropipettor and the solution was vortexed using a Mini Vortex Mixer (LabGenius, London, UK) for approximately 1 min, or longer, until the solution was thoroughly mixed. The resulting solution was then strained through approximately 9 square inches of unbleached muslin cloth[2] into a funnel and collected into a new 20 mL headspace vial (Restek Corporation). Additionally, the solution was manually squeezed via twisting and wringing the unbleached muslin cloth to simulate traditional methods of kava drink preparation. This was done until no additional fluid could be extracted. The resulting strained solution was analyzed. A method blank was also created by vortexing 10 mL of water in a 20 mL headspace vial for approximately 1 min, and then wringing the water through the unbleached muslin cloth into a new headspace vial. The method blank was injected multiple times throughout the sequence to verify the absence of carryover between samples.

Table 1. List of kava samples analyzed in this study[3]

SAMPLE #	NAME	BRAND	PURPORTED ORIGIN	PURCHASED
1	Kava Lateral Root	Kava King	Vanuatu	Honolulu
2	Hawaiian Kava Powder	Maui Medicinal	Hawaii	Honolulu
3	Fiji Kava	Saidaya Imports	Fiji	Honolulu
4	Traditional Kava	Waialeale Kava Source	Vanuatu	Honolulu
5	Happy Warrior	Waka Kava	Fiji	Honolulu
6	Kava Tonga	Tikaram's	Tonga	Honolulu
7	Vanuatu Kava	Tikaram's	Vanuatu	Honolulu

[2]Unbleached muslin cloth was chosen because it is used by some contemporary indigenous practitioners in Hawai`i to filter kava preparations

[3] Though all kava packages indicated a place of origin of the kava (e.g., Vanuatu), the authors did not have a way of independently verifying origin. The phrase “purported origin” is meant to indicate this uncertainty, but is not meant to suggest that sellers were attempting to mislead consumers. Importers are well aware of the challenges of quality control and proof of the origins and identity of the kava they import.

8	Uno'ho	Uno'ho	Tonga	New Zealand
9	Pure Kava (Waka) Powder	Tikaram's	Fiji	Honolulu

2.3. SPME Arrow sampling

Solid Phase Microextraction Arrow sampling with a polydimethylsiloxane (PDMS)/carbon wide range (CWR) fiber (Restek Corporation) was performed using 20 mL headspace vials (Restek Corporation) containing approximately 1 g of kava root powder or the extracted water sample. Sample extraction and injection was performed using a TriPlus RSH Autosampler (Thermo Scientific, Waltham, MA, USA).

Sample incubation and extraction were performed at 35 °C for 5 min, at 500 rpm and 1000 rpm respectively. The needle speed was 20 mm/s in the vial and the needle depth was set to standard. The incubation mode was constant. Injection was performed to a depth of 45 mm, with 35 mm/s penetration speed for 1 min. The GC was set to start when the needle entered the injector. SPME Arrow fibers were conditioned for 30 min at 260 °C prior to each sequence and were confirmed blank with a fiber blank injection. SPME Arrow fibers were reconditioned with a pre-desorb time of 2 min and a post-desorb time of 5 min in between each run.

2.4. GC×GC-qMS/FID method

The method for analyzing VOCs from kava samples using GC×GC-qMS/FID was originally published in Byrne et al. (2020) and is described below. The instrument was a Trace 1300 GC/FID and an ISQ 7000 Single Quadrupole Mass Spectrometer (Thermo Scientific). The inlet was operated in split mode with a split flow of 20 mL/min and a purge flow of 5 mL/min. The inlet temperature was 250 °C. The first dimension column was an Rxi-624Sil MS column (30 m x 0.25 mm ID x 1.4 µm film thickness, Restek Corporation) and the second dimension column was a Stabilwax (5 m x 0.25 mm x 0.25 µm film thickness, Restek Corporation). An INSIGHT Reverse Fill/Flush Modulator (SepSolve Analytical, Peterborough, UK) was used with a modulation period of 2.5 s and flush time of 100 ms. The loop dimensions were 0.53 mm ID × 1133 mm with a resulting loop volume of 25 µL. The bleed line dimensions were 5 m × 0.1 mm ID. Ultra high purity helium (Airgas, Radnor, PA, USA) was used as the carrier gas. The flow rate in the first dimension column was 1.00 mL/min and the auxiliary gas flow rate was 20.00 mL/min, with a calculated flow rate of 17.9 mL/min in the second dimension. The flow was split between the FID and MS using an unpurged SilFlow GC 3-port splitter (Trajan Scientific and Medical, UK) at approximately 4.5:1, which was maintained constant throughout the run. An uncoated fused silica column was used to connect the splitter device to each of the FID and MS. The GC oven started at 60 °C, was held for 1 min, increased to a final temperature of 250 °C at the rate of 5 °C/min, and held for 10 min. The total run time was 49 min.

The ion source temperature and the transfer line temperature for the qMS were both set to 280 °C. The qMS was operated in electron ionization mode with a scan range from 40-300 *m/z.* The total scan time was 0.0241 s, which resulted in an acquisition rate of 41.5 scans/s. This is the maximum acquisition rate for this instrument when using this scan range. Prior publications (Dubois et al., 2020) provide the resulting specifications on data acquisition from this particular

setup. The FID was operated with 350 mL/min ultra-zero grade air (Airgas), 40 mL/min ultra high purity nitrogen as makeup gas (Airgas), and 35 mL/min ultra high purity hydrogen (Airgas). The temperature of the FID was set at 250 °C. The scan rate of the FID was 120 Hz. Instrument control was performed using Chromeleon 7 version 7.2.9 (Thermo Scientific).

2.5. Data processing

Data acquired in Chromeleon was exported to *.raw files for qMS data, and *.cdf files for FID data. The qMS files were then converted to *.cdf in File Converter. The *.cdf files were then imported into Chromspace v. 1.4 (SepSolve Analytical). In Chromspace, the MS contour plots were used to tentatively identify compounds and create a set of stencils that was then transferred to the FID data for quantification. Baseline correction was performed using 0.35 s.

In Chromspace, the stencils containing tentatively identified compounds were generated after the MS contour plots were integrated and compounds were added from the area percent table. Integration used a curve fitting algorithm with 3 Pseudo Gaussian smoothing points. Overlap was set to 2%, correlation was set to 0.3, tolerance was set to 2%, and intensity was set to 2%. Fronting and tailing values were set to 2% for high and low modulation periods (P_M). The absolute minimum peak height was set to 250,000. The search library peaks function was selected which was linked to the National Institute of Standards and Technology (NIST) 2017 Mass Spectral Library. Compounds that had a reverse match factor (RMF) of 800 or above were included from the area percent table. The stencil was saved and loaded onto new samples after baseline correction and integration and new compounds were added until the stencil was finalized.

Once finalized, the stencils generated from the MS contour plots were transferred to the FID data for quantification. Baseline correction was performed using 0.35 s. The stencils were adjusted using the alignment and region tools to adjust the compound boxes over the correct peak. Once the stencil was adjusted, the FID contour plots were integrated. Integration used a curve fitting algorithm with 3-point Pseudo Gaussian smoothing. Overlap was set to 2%, correlation was set to 0, tolerance was set to 2%, and intensity was set to 0.5%. Fronting and tailing values were set to 2% for high and low P_M. The absolute minimum peak height was set to 0. Local region of interest was selected. In the area percent table, “TIC” was deselected and the tentatively identified compounds in the stencil were included. Identification and Calibration functions were activated and the method was saved. The FID contour plots were sequenced in Chromspace using the methods generated for the samples. Sample Summary Reports containing peak tables were generated. A prior publication (Byrne et al., 2020) provide the data processing workflow for non-targeted analysis of VOC data described above.

The peak tables generated from the sequenced FID data were exported into a Microsoft Excel (2016) file (*.xlsx) to perform alignment of the compounds. The sample summary report for each sample was exported and the peak areas from individual sample summary reports were then combined into the same excel spreadsheet. The peak area values for each sample were weighted based on the exact reported weights of the dry kava powder, to allow peak areas to be normalized to amount of sample. The data were exported into The Unscrambler X version 10.4

(CAMO Software, Oslo, Norway) to perform Principal Component Analysis (PCA). The data were treated by mean centering, scaling to standard deviation, and unit vector normalizing the peak table after the values were weighted.

3. Results

The headspace above the different kava samples was analyzed in order to characterize the VOC profile from each. The headspace was investigated from both the root powder as originally purchased and by performing a scaled-down extraction into water mimicking traditional preparation of the beverage. Figure 1 displays GC×GC contour plot examples of the kava powder and extract VOC profiles obtained, and demonstrates the complexity of the profiles with many compounds present. Some compounds of interest are also highlighted. Plots of the powder and extract VOC profiles were obtained for each of the nine different kava samples. A summary plot of compound location is provided in the supporting information in Figure S1.

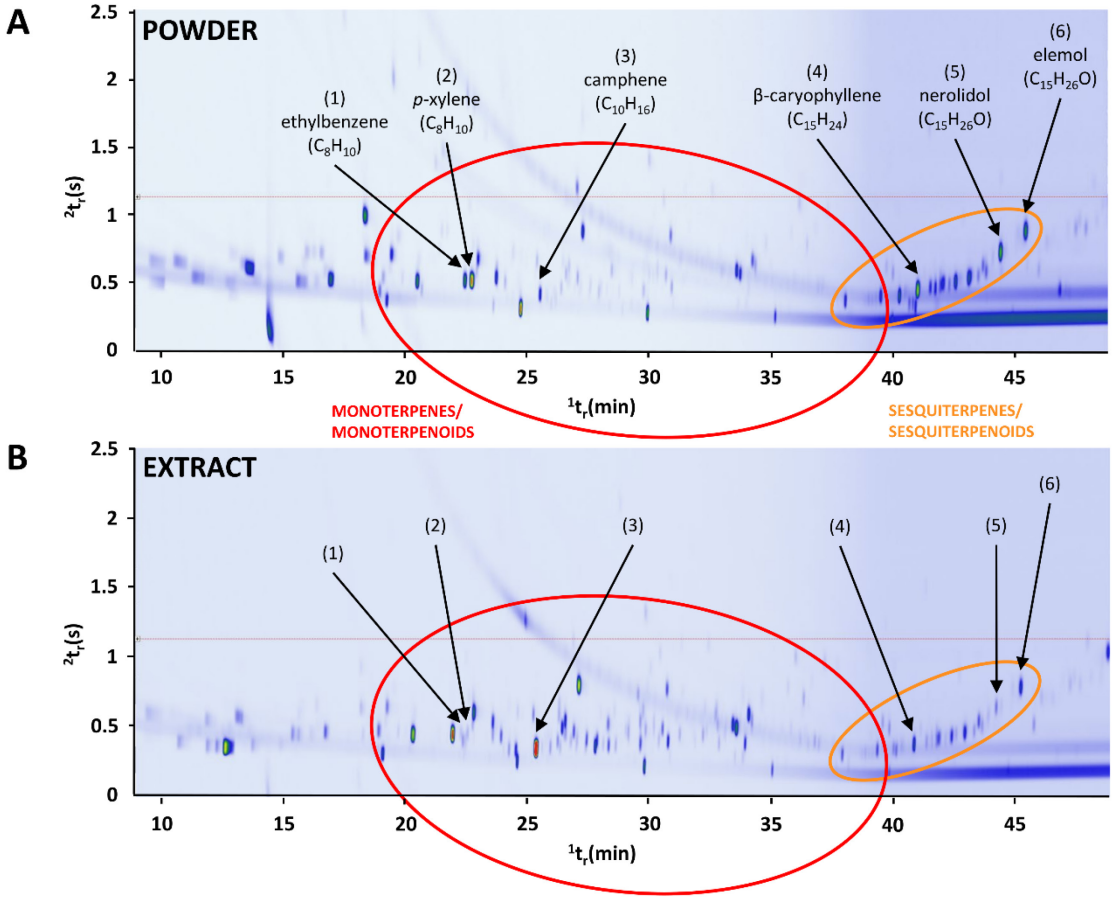

Figure 1. Comprehensive two-dimensional gas chromatography (GC×GC) contour plots. Plots were generated from the flame ionization detection (FID) data stream for Waka Kava brand kava in **(A)** powder and **(B)** extract forms. The region circled in red shows the area in which monoterpenes/monoterpenoids eluted, in addition to other components, with some compounds of

interest highlighted. The region circled in orange highlights the area in which sesquiterpenes/sesquiterpenoids eluted, in addition to other components.

The PCA scores plot in Figure 2 shows the separate clusters formed for each of the kava powdered samples and water extracts based on the compounds detected in each of the VOC profiles. Tikaram's brand was the only supplier from which products were obtained across three different purported origins, and therefore were of interest in better understanding potential regional differences. Figure 2 shows that the three kava samples from the Tikaram's brand (Samples # 6, 7, and 9) clustered together in each of the overall powder and extract groups. These three samples clustered based on their brand origin, rather than being differentiated with other kava samples purported to be from similar region origins.

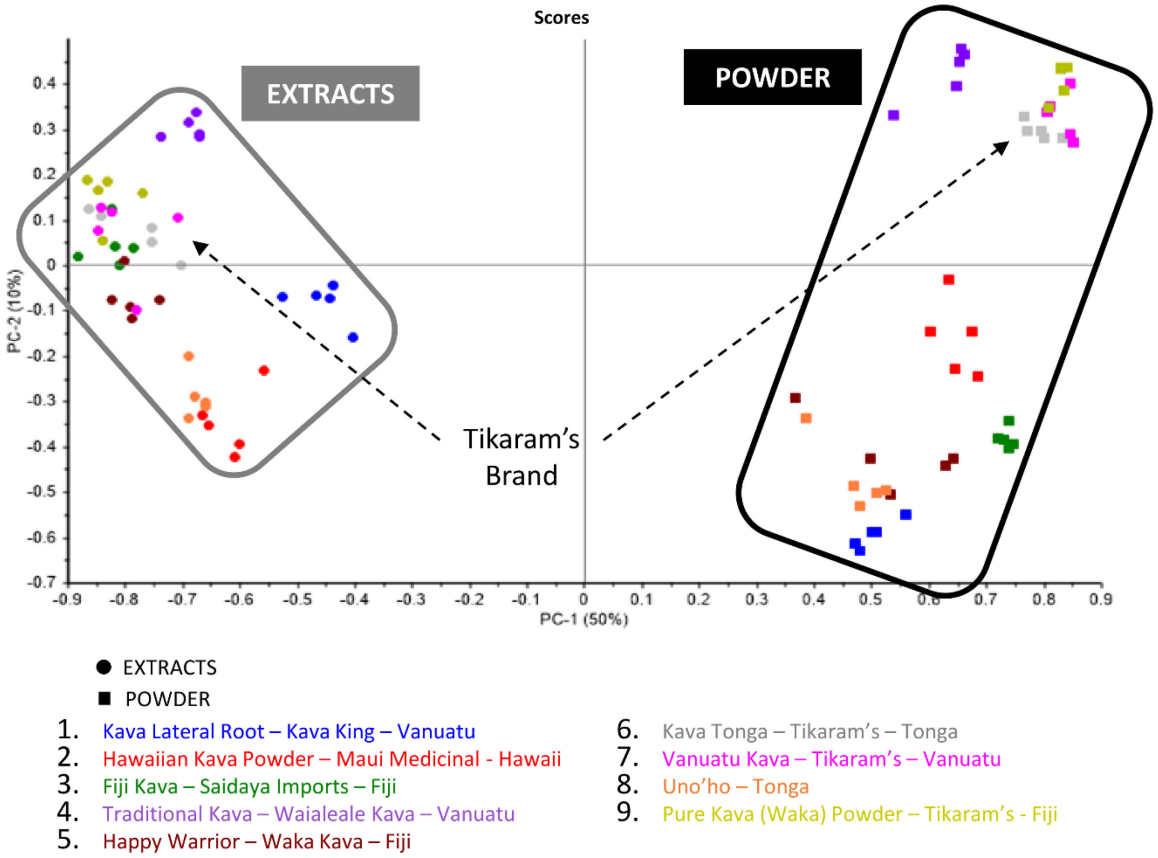

Figure 2. Principal component analysis (PCA) scores plot for all samples. PCA plots illustrate sampling clusters from all samples of kava powder and extracts.

The three Tikaram's brand kava samples from different location origins were analyzed and the following PCA scores and loadings plots were generated (Figure 3). Presented in Figure 3A as expected, separate clusters formed for the powdered samples and water extracts. The variables shown in the loadings plot (Figure 3B) correlate to compounds detected from the powder and extract kava samples. The numbers associated with each variable correspond to the designated compound number given based on the complete list of VOCs detected from the kava samples, presented in supplementary Table S.1. Both the PCA scores and loadings plots indicate there is

less variation in the compounds found in the Tikaram's powder, and more variation in the compounds found in the Tikaram's extracts. The loadings plot further illustrates that certain compounds were generally more likely to be found in the powder and other compounds more likely in the water extracts. Before extraction, the powder samples from different regions clustered close together and appear quite similar to each other. In comparison, after extraction more differentiation was observed between the different purported origins. In powder form, the location region of the powders did not seem to make a difference, whereas there appeared to be some degree of clustering along the second Principal Component after extraction from samples of the same purported origin. Examples of some compounds influencing the Tikaram's brand kava powder clustering were 2-ethyl-1-hexanol, 1-pentanol, acetic acid, acetoin, ᴅ-limonene, and nerolidol. These compounds were either only present in powder form, or present at a fraction of the abundance in extract compared to powder form. The relationship with the compounds associated with the powder samples appeared to be consistent regardless of whether they originated from Fiji, Vanuatu, or Tonga. For extracted Tikaram's samples, certain compounds influenced clustering based on purported region. Examples of some compounds that influenced the cluster of Tikaram's Tonga extracted samples were ethyl ether, and 1,4-dimethyoxy benzene. Some compounds that influenced the cluster of Tikaram's Fiji extracts were σ-cadinene, β-elemene, α-muurolene, β-myrcene, and β-phellandrene. Examples of compounds that influenced the cluster of Tikaram's Vanuatu extracts were benzaldehyde and hexanal. The family of terpene compounds was present in higher abundance in the cluster of Tikaram's water extracts from Fiji, compared to extracts from Tonga or Vanuatu.

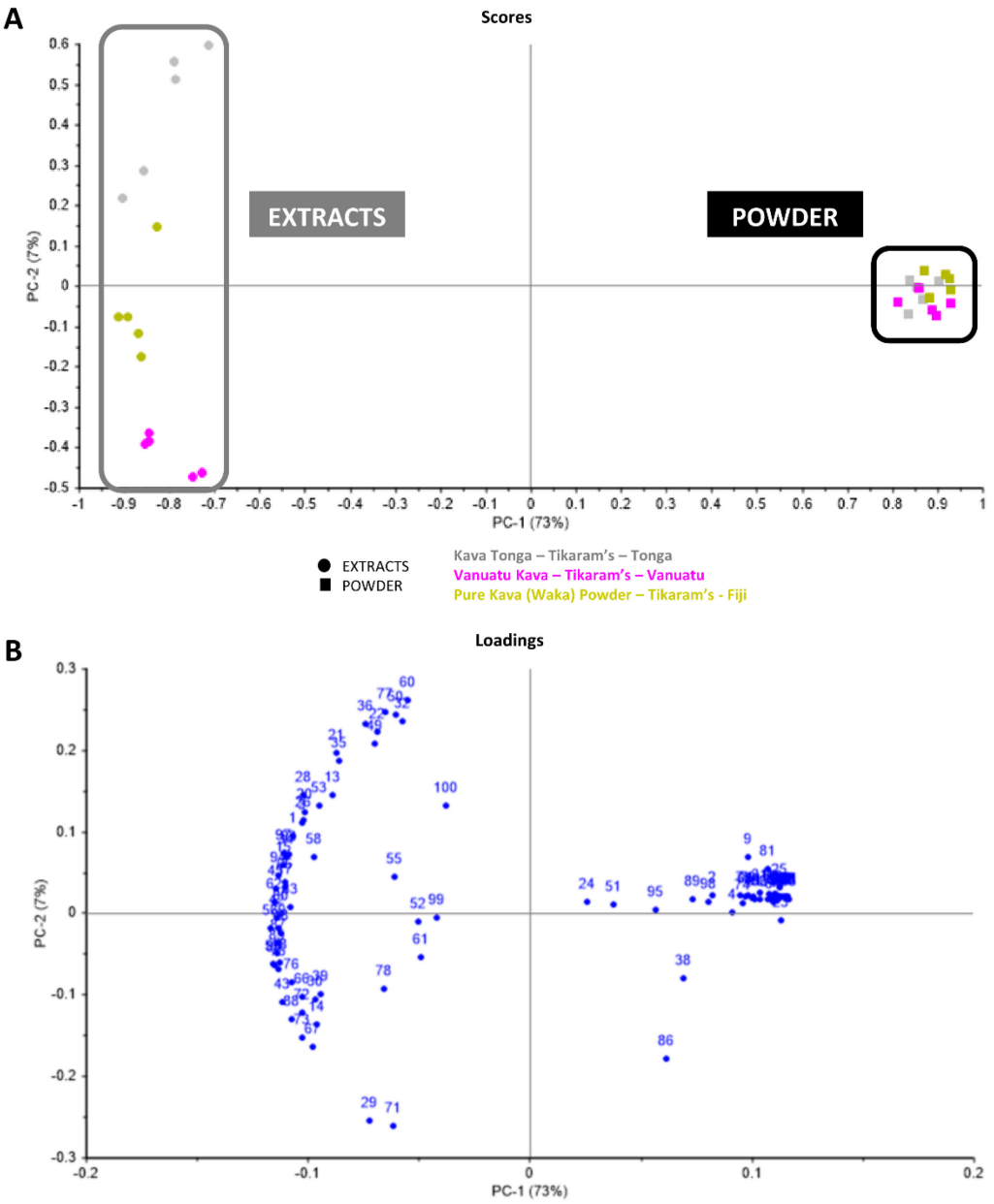

Figure 3. Plots for Tikaram's brand samples. Principal component analysis (PCA) (**A**) scores plot and (**B**) loadings plot generated from Tikaram's brand of kava powder and extracts.

It should be noted that purported origin was not verified in this study. The intention was to collect a range of commercially available samples of dried, powdered kava roots from different origins to represent a range of profiles from kava products. Samples were labelled by purported origin in the results, but with the intention of investigating similarities in the profiles between samples rather than to determine if different regions produce different profiles. Further work could investigate regional profiles by analyzing samples with verified provenance from field to laboratory, and performing discriminant analysis to improve understanding of what differentiates kava grown in different regions.

The supporting information (Table S.1.) demonstrates the full table of compounds detected in this study with a total of 99 VOC compounds from all sample types, after removing methodological and instrumental artifacts from the components detected. Table 2 exhibits a condensed summary table including the VOCs present in both powder and extract forms, and the top compounds tentatively identified in powder and extract form. The table includes hydrocarbons, terpenes, terpenoids, alcohols, aromatic compounds, aldehydes and ketones. The GC×GC-qMS/FID instrumentation was powerful in resolving this large number of components with a wide range of functional groups, and demonstrated the power of separation achieved in comparison to traditional approaches using techniques such as one-dimensional gas chromatography (1D GC). The separation structure of the plots (i.e., Figure 1) also allowed an improved ability to differentiate between isomers with very similar chemical structure that cannot traditionally be distinguished using 1D GC with quadrupole mass spectrometry.

Table 2. Condensed list including 38 of 99 volatile organic compounds (VOCs) detected in kava samples. Compounds included are those present in both powder and extract form, and those of highest abundance in either form. VOCs were analyzed using comprehensive two-dimensional gas chromatography – quadrupole mass spectrometry/flame ionization detection (GC×GC-qMS/FID). Tentative analyte identifications were made using qMS data and reported retention times are based on FID data.

Designated Compound #	COMPOUND NAME	Molecular Formula	MW (g/mol)	RI	$^{1}t_R$ (min)	$^{2}t_R$ (s)	Present in POWDER	Present in EXTRACT
3	1-hexanol	$C_6H_{14}O$	102.16	2072	22.77	0.30	X	X
9	1-pentanol	$C_5H_{12}O$	88.15	1688	18.15	0.29	X	X
20	3-hexen-2-one, 6-phenyl-	$C_{12}H_{14}O$	174.24	--	44.46	0.09	X	X
22	3-phenylpropanol	$C_9H_{12}O$	136.19	3537	36.16	0.60	X	X
23	acetic acid	$C_2H_4O_2$	60.05	1320	14.10	2.39	X	X
25	acetoin	$C_4H_8O_2$	88.11	1686	18.11	0.64	X	X
26	acetophenone	C_8H_8O	120.15	2986	30.81	0.48	X	X
27	α-amorphene	$C_{15}H_{24}$	204.35	--	42.65	0.09	X	
29	benzaldehyde	C_7H_6O	106.12	2661	27.16	0.52	X	X
30	benzaldehyde, 4-methoxy-	$C_8H_8O_2$	136.15	3578	37.36	0.29	X	X
32	benzene, 1,4-dimethoxy-	$C_8H_{10}O_2$	138.16	3255	33.17	0.41	X	X
34	benzylacetone	$C_{10}H_{12}O$	148.20	3545	36.37	0.32	X	X
35	benzyl alcohol	C_7H_8O	108.14	2955	29.94	0.92	X	X
38	bornyl acetate	$C_{12}H_{20}O_2$	196.28	3564	36.93	0.06	X	X
41	β-cadinene	$C_{15}H_{24}$	204.35	--	43.19	0.22	X	
43	camphene	$C_{10}H_{16}$	136.24	2387	25.39	0.07	X	X
45	ι-camphor	$C_{10}H_{16}O$	152.23	3266	33.48	0.13		X
46	α-caryophyllene	$C_{15}H_{24}$	204.36	--	42.14	0.10	X	
47	β-caryophyllene	$C_{15}H_{24}$	204.36	3886	41.08	0.07	X	X
48	copaene	$C_{15}H_{24}$	204.36	3798	38.06	2.40	X	X
55	(-)-β-elemene	$C_{15}H_{24}$	204.35	3841	39.52	0.01	X	

56	elemol	$C_{15}H_{26}O$	222.37	--	45.56	0.39	X	X
57	endo-borneol	$C_{10}H_{18}O$	154.24	3284	33.98	0.23		X
59	ethylbenzene	C_8H_{10}	106.16	2056	22.26	0.15	X	X
61	β-farnesene	$C_{15}H_{24}$	204.36	3864	40.31	0.17	X	
63	furan, 2-pentyl-	$C_9H_{14}O$	138.21	2415	26.26	0.11	X	X
69	hexanal	$C_6H_{12}O$	100.16	1997	20.28	0.17	X	X
70	α-ionene	$C_{13}H_{18}$	174.28	--	43.79	0.12	X	X
73	ι-limonene	$C_{10}H_{16}$	136.23	2677	27.64	0.02		X
74	linalool	$C_{10}H_{18}O$	154.25	2979	30.61	0.19	X	X
77	β-myrcene	$C_{10}H_{16}$	136.23	2405	25.95	0.01	X	X
78	nerolidol	$C_{15}H_{26}O$	222.37	--	44.51	0.23	X	X
82	oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	151.16	2375	25.02	0.79	X	X
83	pentanal	$C_5H_{10}O$	86.13	1645	16.67	0.18	X	X
90	styrene	C_8H_8	104.15	2330	23.59	0.24	X	X
91	sulcatone	$C_8H_{14}O$	126.19	2654	26.94	0.19	X	X
96	valencene	$C_{15}H_{24}$	204.36	--	41.61	0.01	X	X
97	*p*-xylene	C_8H_{10}	106.16	2065	22.55	0.15	X	X

Figure 4 provides an overall summary of the VOCs detected in the kava powder and water extracts. The Venn diagram (Figure 4A) illustrates the distribution of the compounds. The dry root samples contained a total of 68 compounds and the water extracts contained 61 compounds. Of the 68 and 61 compounds detected in each group, 30 of the compounds were shared between the powder and extract kava samples. The top 10 compounds, based on overall average abundances, for each of the kava powdered and extracted samples were compared, shown in Figure 4B and 4C. While dry root samples contained high levels of β-caryophyllene, water extracted samples showed high levels of camphene. β-caryophyllene was also present as a top compound in the kava extracts, but at 10% of the amount present in powder form. Camphene was also present as a top compound in the kava powder, but also only at 10% of the amount present in the extracted form.

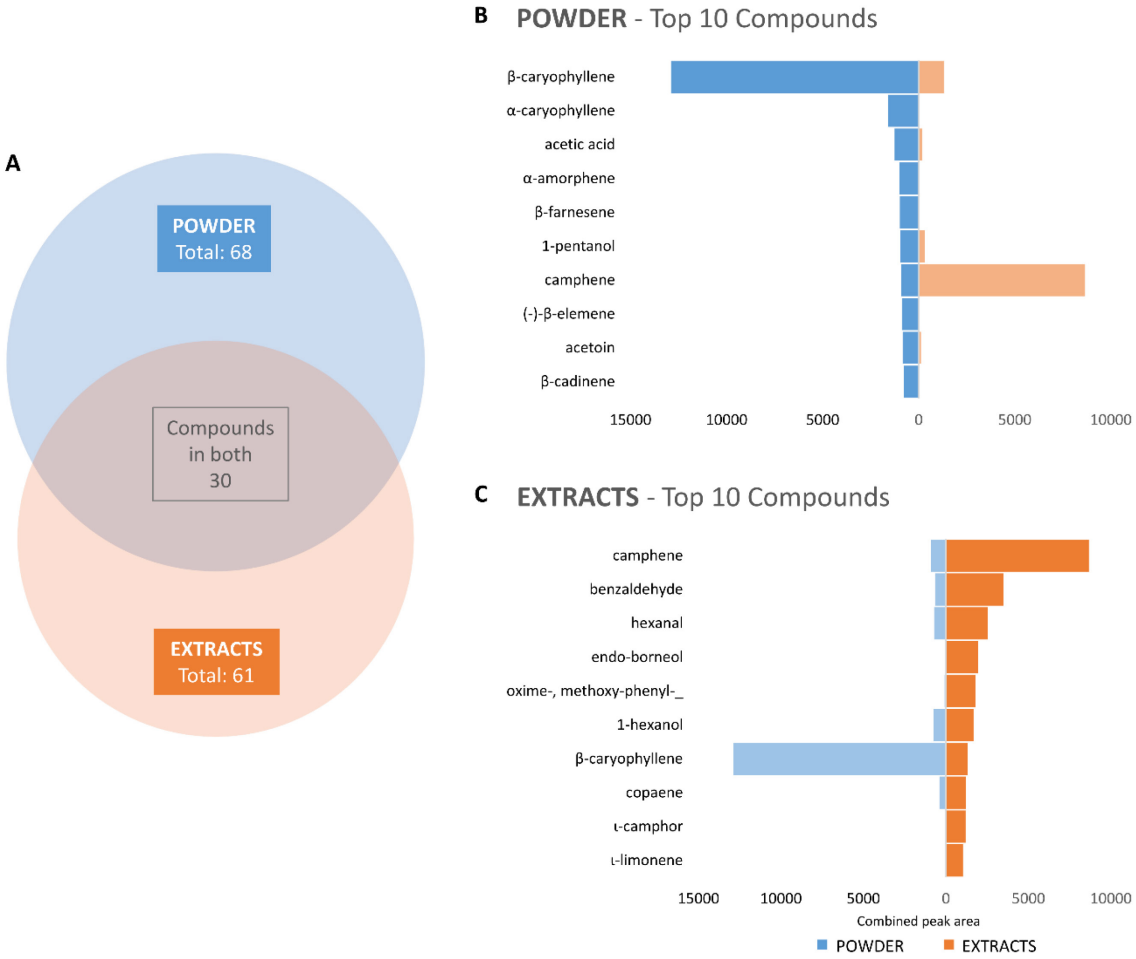

Figure 4. Summary diagrams of overall volatile organic compounds (VOCs) detected. **A)** Venn diagram of total VOCs detected in kava powder and extract samples. Compound comparisons of top 10 compounds with highest overall average abundances across all kava **B)** powder and **C)** extract samples.

Terpenes and terpenoids were both present in the list of top extracted compounds. Terpenes were found consistently in both powder and extracted samples, but the distribution of terpenes differed by sample type. For example, caryophyllene had notably higher abundance in powder samples whereas camphene had notably higher abundance in extracted samples. Figure 5 demonstrates select compounds in order from most to least abundant for major compounds in each class. Terpenoids are relatively more polar and therefore would be expected to extract better in water comparatively. The top terpenes found in kava extracts were camphene, caryophyllene, copaene, and limonene. The top terpenoids in extracts were linalool, endo-borneol, and camphor. Additional major compounds found in water extracts included benzaldehyde, hexanal, methoxyphenyloxime, 1-hexanol, and elemol. The compounds that contributed to the VOC kava profiles were classified into major chemical classes: alcohols, aldehydes/ketones, aromatics, terpenes, terpenoids, and other. Comparison of the different chemical classes of compounds detected in the powdered kava samples versus the extracts is displayed in Figure 5. This plot shows selected compounds that were detected in each class. Out of 99 total VOC compounds

discovered, 34 were terpene or terpenoid compounds. Figure 6 shows that the classes of terpenes and terpenoids together represented a larger abundance than the other chemical classes.

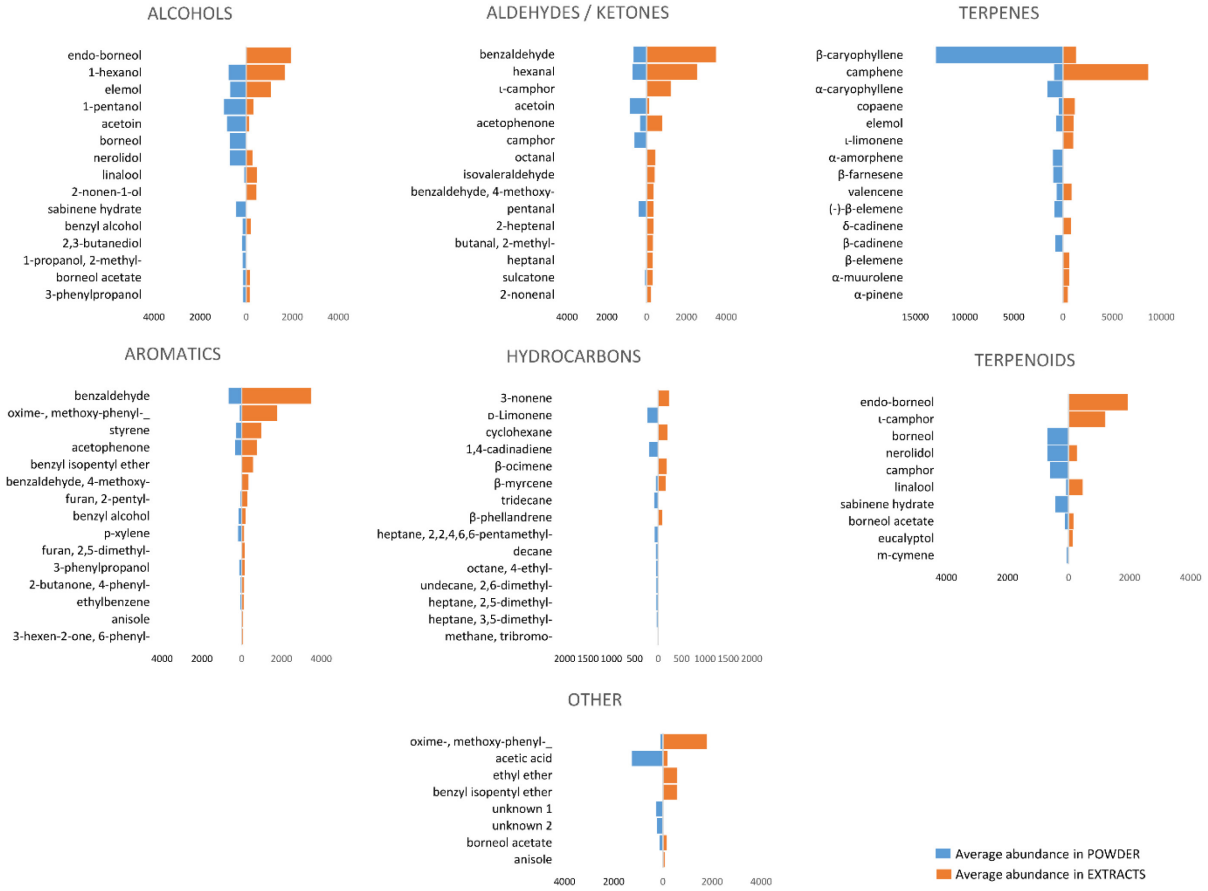

Figure 5. **Kava volatile organic compound (VOC) comparisons between major chemical class groups.** Comparison of average abundances for the 7 different major chemical classes of VOC compounds detected in kava powder vs extracts. Select compounds are displayed.

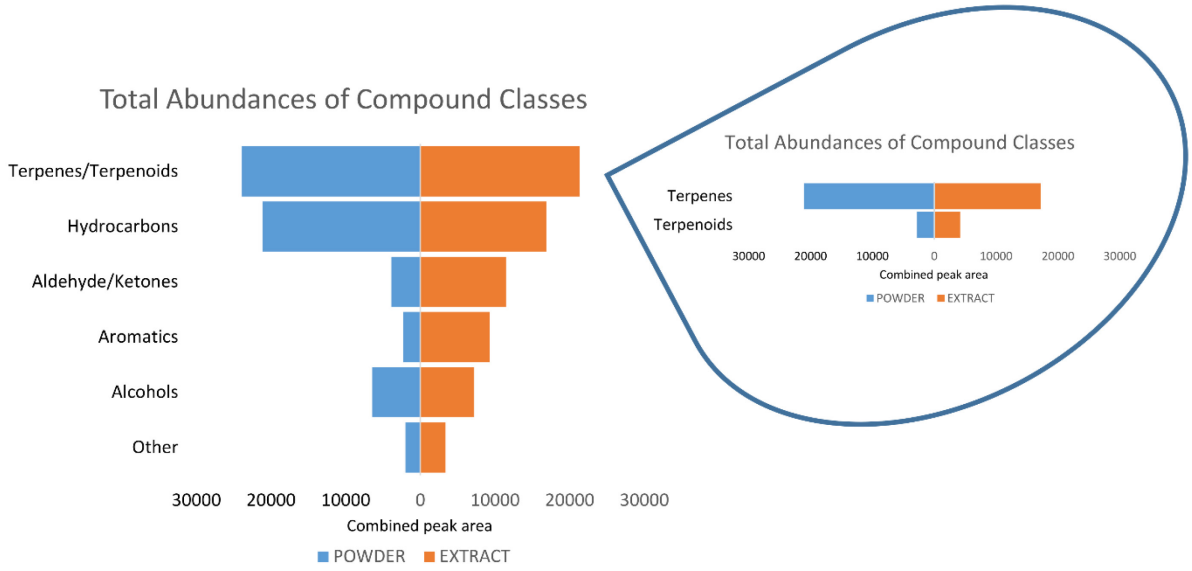

Figure 6. Overall comparison of major chemicals classes of kava volatile organic compounds (VOCs). Overall total abundances of each type of VOC compound class detected in kava powder vs extracts.

4. Discussion

4.1. Kava chemical profile

As a plant product, it is not surprising that the chemical profile of kava is complex. Though prior characterization of kava provides a comprehensive understanding of the kavalactone fraction of this plant material (Lebot et al., 1997; Shimoda et al., 2015; Showman et al., 2015; Singh, 2004; Xuan et al., 2008), there is minimal research being conducted on other components of the kava metabolome. Attention was brought towards this point first by Xuan et al. (2008), where extraction of kava into different solvents yielded different chemical profiles. The authors noted that components other than kavalactones were extracted into different solvents, identifying six non-kavalactone compounds in extracts that had never been previously identified in kava. Since then, despite the knowledge that other components within kava may have pharmacological potential, minimal work has elaborated on the non-kavalactone component of this profile. Most recently Jaiswal et al. (2020) used gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS) to investigate the kavalactone content in lateral roots and crown root peels. They reported a number of non-kavalactone compounds present within sample extracts. They identified 19 non-kavalactone compounds using GC-MS and a further 19 non-kavalactone compounds using LC-MS (Jaiswal et al., 2020). In examining the total ion current chromatograms from this study, it can be observed that there is a significant amount of co-elution occurring in these chromatograms due to the complexity of the profile, meaning that the number of non-kavalactone compounds present may have been significantly underestimated due to masking. The use of multidimensional gas chromatography in this study by way of GC×GC analysis affords improved chromatographic resolution and ability to resolve all components from one another, significantly enhancing the detectability of analytes. As a

result, the study herein reports 99 non-kavalactone components using GC×GC analysis, which represents the most comprehensive examination of the kava secondary metabolome to date. While previous studies establish different fractions of the kava metabolome using different methods (Jaiswal et al. 2020; Xuan et al. 2008), these results contribute significant fidelity to the volatile and semi-volatile constituents found in commercially available kava root, and in root material extracted in water. These results are comparable to work in other *Piper* spp. of economic and therapeutic significance, and they allow for a more in-depth contextualization of the broad ethnomedical uses documented in the ethnographic record (see 4.2 below).

Terpenes and terpenoids are the most abundant chemical class group of VOCs found in both powder and extract kava samples (Figure 6). Terpenes are hydrocarbons and oxygenated terpenes are terpenoids. These are compounds found in plants, herbs, and oils which are associated with pharmacological properties and medicinal benefits (Baron et al., 2018; Cox-Georgian et al., 2019). Terpenes and terpenoids are the source of plant aromas and flavors, and have been found to be naturally occurring in 50-70% of all studied plants (Desaulniers Brousseau et al., 2021). In this study of kava, 34 different terpenes and terpenoids were detected, with camphene found to be extracted in the highest abundance (Figure 4C).

One of the goals of this research was to consider the variation both amongst the samples themselves, and between powdered kava and water extracts. Given that the samples were stated as originating from multiple island groups in the Pacific, the question was whether there would be any correlation between the VOC profile and stated geographic origin. With respect to possible variation between powder and water extracts, the water extracts were performed with the goal of better understanding kava as it is consumed as a beverage. The goal was to explore the chemical components that contribute to the drink profile. This is important since medicinal and social use of kava as a beverage continues in the Pacific and the Pacific Islander diaspora, and since interest in water-extracted kava root preparations is growing in nontraditional contexts such as kava bars (Baker, 2012). Much of the previous work on kava has relied on methods that by their nature may result in extracts that do not adequately mimic what consumers are actually ingesting. While the approach presented here is a first step and could be further developed to improve the fidelity of the linkage between what is being analyzed and what is being consumed, these results are an important step in this direction. These two types of variation are discussed briefly below.

4.1.1 Variation between powder and water extracts

The powdered samples and water extracts clustered as very distinct groups (Figure 2). This indicated that the compounds generally found in kava powders across brands were similar to other powders analyzed, and the compounds extracted in water across brands were similar to other kava extracts analyzed. Furthermore, it appeared that terpenoids (Figure 7B) extracted better in water than the terpene compounds (Figure 7A). When comparing the combined peak area of the medicinal properties from terpenoids (Figure 7B) for the water extracts (orange bars) with the powdered samples (blue bars), the terpenoid compounds were fully extracted and even appeared to be detected to a greater extent in extract form (more about this in section 4.5). On the other hand, when comparing the combined peak area for each medicinal property in Figure 7A

for the terpene compounds, five of the seven medicinal properties for water extracts were present at 70-80% of the combined peak area from the powder. It appeared that the terpenes were not fully extracted. This may suggest that the terpenoids may play a larger role than the terpenes in therapeutic effects from the volatile and semi-volatile constituents of kava prepared with water. Interestingly, terpene compounds associated with antianxiety and antiviral benefits were extracted to an even lesser extent (Figure 7A) compared to the powder terpene total abundance.

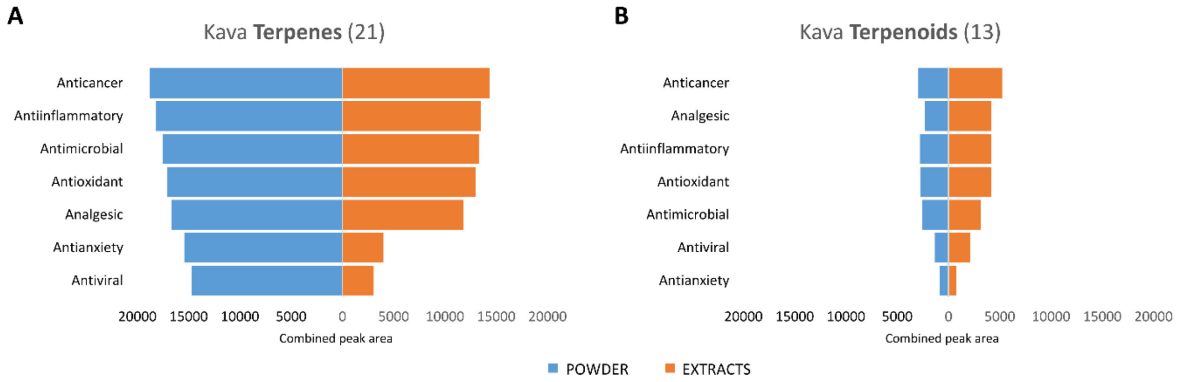

Figure 7. Comparison of kava powder and extracts in relation to terpene and terpenoid compounds and medicinal properties. Total abundances of **A)** terpenes (21 compounds), and **B)** terpenoids (13 compounds) detected in kava, sorted by their associated medicinal properties. Properties were sourced from literature as indicated in Table S.2.

These results indicate an interesting emergent pattern that is worth exploring further. It is likely that water extraction modulates the bioactivity of kava in that it is more selective towards certain compounds over others. Furthermore, the temperature of the water likely also plays a role. In contrast to the preparation of the beverage, made from the roots with ambient temperature or cool water and occasionally coconut water, medicinal preparations vary more widely. Some medicinal water extractions involve boiling; in other preparations, the juice of the leaves is expressed without addition of water (Lebot et al., 1997). The degree of nuance and sophistication apparent in the ethnographic record suggests an awareness of how to manipulate the extraction of these constituents in line with a range of intended outcomes. The VOC data presented here, while preliminary for the purposes of elucidating the subtleties of the wide range of traditional therapeutic kava preparations, are intriguing and suggest avenues for future research.

4.1.2 Variation among samples based on brand and/or stated geographic origin

With respect to variation among samples based on brand or purported origin, the clearest pattern is that the Tikaram's brand samples appeared to cluster together. This is highlighted with the arrows in Figure 2, where the Tikaram's powder samples and extract samples clustered close to each other in their respective groups. This pattern is similar in both the powder and water extracts of these three samples. These three Tikaram's samples are labeled as having originated from three different geographical locations and did not appear to differentiate in the same manner with other brand samples that share similar origins (e.g., the Tikaram's sample labeled as originating from Vanuatu has a VOC profile more similar to the Tikaram's samples labeled as

originating from Tonga and Fiji than it does with other samples purportedly originating from Vanuatu). The trend was confirmed by creating separate PCA visualizations for each individual dataset for samples from Vanuatu and Fiji (data not shown).

Overall, there were no clear correlations between purported geographic origin and VOC profile of powdered kava. With respect to VOC profiles of water extracts, the samples labeled as originating from Fiji (Happy Warrior, Saidaya Imports, Tikaram's) clustered together (Figure 2). Extracts of the other two Tikaram's samples (labeled as originating from Tonga and Vanuatu respectively), also clustered with these (see Figure 3). Given the limited sample size and the lack of definitive knowledge about the origins of these samples, it is important not to overstate the significance of this cluster. Further analysis on a larger range of samples that each have a well-established provenance would better address this question. Yet, since significant kava cultivar diversity exists for every island group where it is grown (Lebot et al., 1997), it is unclear what further investigation would reveal. As it stands, these results are an indication of the variability of VOC profiles of dried kava root samples available in Honolulu at the point in time when they were bought.

Based on the analysis of Tikaram's brand samples in Figure 3, terpenes appeared to be more likely associated with extracts from the samples originating from Fiji. Of the Tikaram's brand samples from different regions, the Fiji-labelled samples had several more terpenes present than the other regionally-labelled samples. In Figure 3 it was noted that terpenes present in the Tikaram's brand samples were also similar to those terpenes seen in the single Fijian kava. It would be interesting to examine whether any of the differences observed among samples of different origins correlate with differences in the physiological effects consumers experience. This is a regular topic of discussion among kava drinkers who are concerned about quality, since variation in the organoleptic properties of different batches of kava can be readily perceived. Additionally, the ethnographic record of therapeutic uses of kava suggests the possibility of a correlation between recognized cultivars and varying effects (Lebot et al., 1999, 1997; Salehi et al., 2019), and this is further supported by the evidence for distinct chemotypes in *P. methysticum* (Lebot et al., 1999, 1997) and other *Piper* spp. used as medicine (Salehi et al., 2019). However, while these results suggest avenues for further investigation of these differences, it is not possible to correlate differences in effect from these results alone.

A study of physiological effects and their correlation to differences in the chemical profiles from kava of different origins would be an interesting avenue for future work.

4.2 Therapeutic implications of terpenes and terpenoids in kava

Terpenes and terpenoids with known medicinal properties account for a third of the VOC profile of kava. Figure 7 shows the relationship of the medicinal properties and the terpenes/terpenoids tentatively found in kava. In this study, the terpene/terpenoid compounds were researched in literature and organized by their broad pharmacological effects (Baron, 2018; Cox-Georgian et al., 2019; de Cássia da Silveira e Sá et al., 2017; Hanuš and Hod, 2020; Nuutinen, 2018). The literature review results attributing pharmacological activity to specific constituents is available as Supplementary Table S.2. The main medicinal properties exhibited by terpenes categorized in

this study were antiviral (13 compounds), antioxidant (23 compounds), antimicrobial (23 compounds), antiinflammatory (26 compounds), anticancer (27 compounds), antianxiety (13 compounds), and analgesic (21 compounds). Some compounds are also classified with more than one pharmacological activity; for example, camphene classifies as an analgesic, antioxidant, anticancer, antiinflammatory, and antimicrobial compound. Figure 7 indicates the terpene compounds as a group contribute to a greater proportion of the pharmacological effects compared to the terpenoid compounds; this is true of the number of different compounds as well as the compound combined peak areas.

While it is beyond the scope of this paper to discuss all the pharmacologically active compounds found in kava in detail, one constituent of note is camphene. Figure 4C demonstrates that camphene was present as the most abundantly extracted compound in the kava samples. Compared to other terpenes, camphene's therapeutic power is its strong antioxidant effect, which has possibility in benefitting certain inflammatory diseases (Kim et al., 2020). Camphene has also been reported to have anticancer and analgesic effects (Cox-Georgian et al., 2019; Quintans-Júnior et al., 2013).

4.3 Correlation between pharmacological properties and ethnomedical uses

The known properties of the terpenes and terpenoids found in kava map onto the ethnographic record of ethnomedical use in interesting ways. The large number of compounds with antimicrobial properties correlate with use of kava for treating urogenital infections, and external wounds and skin conditions. Antimicrobial and antiviral constituents may correlate with ethnomedical use in treating respiratory ailments. Analgesic constituents may correlate with ethnomedical treatments of various forms of pain, and the multitude of compounds with this broad activity suggests possibilities of numerous targets for antinociception. Constituents with antiinflammatory properties may play a role in ethnomedical use in treating all these conditions. In some cases (e.g., antimicrobial, antioxidant, analgesic, antiinflammatory, anticanver, and antianxiety properties), the known effects of the VOCs in kava are similar to those of the kavalactones (Bian et al., 2020; Lebot et al., 1997; Singh, 2004), whereas in other cases (e.g. antiviral), these properties extend beyond the known effects of kavalactones.

An exploration of other species in the genus *Piper* further supports the argument that VOCs in kava, above and beyond the kavalactones, contribute to its various therapeutic applications. The rationale for this is that: (1) many species in the genus share similar volatile chemistry with *P. methysticum*; (2) they do not contain kavalactones; (3) many are also important therapeutically in cultures outside of Oceania; and (4) there are striking parallels between the traditional therapeutic uses for *P. methysticum* in the Pacific and traditional medicinal uses for other *Piper* spp. in different regions. A review of the chemistry and traditional medicinal uses of 106 *Piper* species worldwide lists 51 species in addition to *P. methysticum.* Traditional uses noted were for pain, including 16 for arthritis pain or rheumatism, 12 for toothache, and 11 for headache. Out of the 51 species, 31 are listed for use treating urogenital conditions, including 13 species specifically used for venereal diseases and urinary tract infections. In addition, 26 species are listed as being used to treat coughs and/or asthma and nine species are used specifically for treating cancers (Salehi et al., 2019). Plants used to treat skin conditions and wounds, and

inflammation (difficult to distinguish from analgesia in the ethnographic record) are widespread in this sample as well. This dataset has its shortcomings, and the ethnographic details about traditional uses are brief. However, the larger pattern of a correlation between traditional uses and volatile constituents with known medicinal properties, distributed across a range of species in different cultures, suggests the possibility of a convergent process of identifying efficacious plants for these conditions. To better explore this idea, a larger dataset is needed.

While the *Piper* species are promising avenues for exploring the possibility of convergence on related VOC profiles for similar therapeutic effect, many of these terpenes and terpenoids are distributed widely in other plant species employed in traditional medicines. For example, previous research looking at analgesia, using a data-mining approach looking at convergences across multiple domains (different cultures and medical systems, plant preparations, ingredients, constituents in those ingredients, and known activities and mechanisms of action) found that β-myrcene has been included as a component of analgesic oils in disparate cultural contexts globally, and in Traditional Chinese Medicine and Japanese Kampo formulations used for pain (Jansen et al., 2019). This suggests that it has been convergently arrived at in numerous traditional medical systems for use as an analgesic. Including the VOC profile data presented in this paper, along with the previously published data on other *Piper* species, in this larger convergence analysis would be a promising avenue for further exploration and may reveal patterns across multiple ethnomedical practices that could in turn inform the choice and use of medicines to meet the needs of those who rely on plants as their primary source of medicine.

4.4 Multi-constituent medicines, additive effects, and synergy

The data presented here elucidate some of the complexity of medicines created from natural products. Rather than see this complexity as impeding an understanding of the mechanism of action of a single primary bioactive constituent, the argument being made here is that the effects of a multiplicity of bioactive compounds might collectively contribute to the therapeutic effects of kava. The complex interactions, either additive, synergistic, or both, among the range of active ingredients may contribute more to the medicinal effects of kava than any single constituent on its own.

This perspective finds support from multiple directions. First, the six main kavalactones found in kava have both additive and synergistic effects. There is a widespread recognition that the physiological effects of drinking kava derive from the total amount and relative proportion of the six major kavalactones – an additive effect (Lebot and Levesque, 1996). Furthermore, there is evidence of synergism among the kavalactones as well. For example, a greater degree of anticonvulsant activity was measured from administration of a mixture of kavalactones than from comparable doses of any single kavalactone (Klohs et al., 1959; Meyer, 1979). This paper builds on this previous recognition of the complexity of the pharmacology of kava by arguing that the VOCs present in kava likewise also may contribute to its recognized bioactive effects and should be further investigated.

Second, in support of further exploration into the bioactive properties of VOCs found in kava, terpenes and terpenoids have been at the forefront of studies in cannabis regarding their

synergistic or entourage effects with cannabinoids (Hanus 2020, Baron 2018, Baron & Lucas 2018). Although terpenes and terpenoids are generally present in relatively low concentrations (in cannabis and kava both), entourage-like effects of a combination of moderately active compounds affecting multiple target sites and pathways may be an explanation for observed therapeutic outcomes in areas such as analgesia or treatment of inflammation that, to date, have not successfully been shown to correlate to the actions of any one single constituent. However, separating the potential of additive or synergistic medicinal activities from the hype associated with unsupported claims used to market proprietary medicines (as is the case for cannabis) is important. The argument here is merely that the convergence of ethnomedical patterns of use and known therapeutic properties of VOCs found in *Piper* spp. warrants further research.

Finally, though this is not meant to downplay the profound significance of the western scientific medical approach of focusing on single isolated active constituents, or the benefits derived from this approach, it is worth noting that most medical practices, including those that are professionally organized and integrated into modern state-overseen medical systems (e.g., Traditional Chinese Medicine, Japanese Kampo, Ayurveda), rely on complex multi-ingredient mixtures made primarily of plants. The western approach struggles to adequately characterize this complexity, and at worst risks rejecting any potential benefits through a process of oversimplification and reduction (Jansen et al., 2021). The argument here is that further exploration of the VOCs in kava may result in a more accurate characterization of the therapeutic intent of those using it as a medicine, and the sophistication underlying those ethnomedical practices.

4.5. Methodological discussion and future directions

The Venn diagram in Figure 4A indicates that the distribution of the 99 total VOCs detected from all kava samples is approximately 1:1:1 between the compounds found in powder only, extract only, and shared between both sample types. The PCA plot of all samples (Figure 2) illustrates that overall, regardless of brand or origin of the kava, the compounds characterized in a powdered sample were consistently found in other powdered samples, and the compounds found in water extracts were consistent in other water extracts. This is further highlighted in the mirror bar comparisons in Figure 5, where the compounds found in the extracts (orange) were not necessarily the same compounds found in the powder form (blue). While some compounds are present in both sample types, it appears that some compounds discovered in the extracted samples were present in relatively low amounts in the original powder form, or not detected at all. Figure 8 displays an illustration of headspace vials containing kava samples from this study in powdered or extracted form. In this study, the SPME needle was injected into the headspace for sampling. It is plausible that volatile analytes are more readily available for headspace analysis in a liquid extraction form as the vial is heated and shaken in preparation for sample injection, and therefore, we may not be fully capturing the kava profile in powder form.

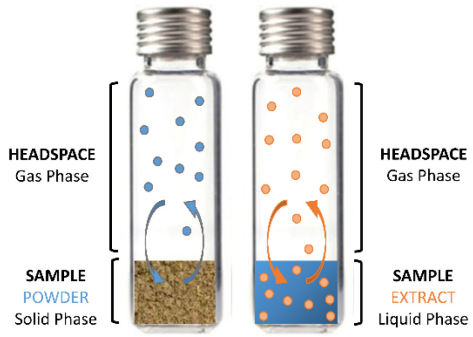

Figure 8. Illustration of volatile organic compound (VOC) dynamics inside of headspace vials. Examples of headspace vials containing powdered versus liquid extract samples.

Immersion of the SPME fiber into the liquid sample would be a good idea for future work to observe the compounds dissolved in the water extract, as a way to perhaps create a more complete extract profile. In this scenario, it is possible that more semi-volatile compounds would be detected rather than volatile compounds. It may also be beneficial to analyze the samples using two-dimensional liquid chromatography (2DLC) for better visibility of semi-volatile components as well. One thing to also note is that this study only extracted the kava samples with water. There are several methods of extracting kava which can vary traditionally from region to region, and may influence the pharmacological effects produced upon ingestion or topical application. While this study does not provide a complete kava profile, employing GC×GC has allowed the possibility of capturing the most comprehensive profile reported on kava so far. The power of multidimensional chromatography is available for these types of profiles, with its ability to analyze a complex mixture that would otherwise be challenging to resolve. For example, a recent study (Oswald et al., 2021) discovered a new family of sulfur components that contribute to the “skunk-like” aroma of cannabis. While terpenes and terpenoids have been studied extensively in regards to plant and cannabis aroma, this new finding of sulfur compounds was not previously possible without GC×GC techniques. Therefore, investigating further into kava sampling could lead to an opportunity to build a more complete kava profile and its potential health benefits.

5. Conclusion

The use of GC×GC-qMS/FID provided valuable data while analyzing the volatile and semivolatile compound profile of kava. While previous research of kava has focused primarily on kavalactones and flavokawains, this research focused on different classes of compounds including terpenes, terpenoids, alcohols, aldehydes, ketones, and aromatics, thus enabling a broader range of compounds from the secondary metabolome of kava to be investigated. This research analyzed kava in a dry powder form and when extracted into water, in order to mimic how it is traditionally prepared. This data will assist in understanding the transfer of compounds from kava powder into water when it is extracted and consumed in this manner. After sample analysis and data processing, a clear distinction was observed between the dry kava powder and the kava extract samples. While samples obtained from the same brand also clustered together, samples did not cluster together based on purported origin. A total of 99 compounds were

tentatively identified from both dry kava and extract samples with 68 compounds from the powder, 61 compounds from the extracts, and 30 compounds found in both types of samples. A number of compounds in both sample types were found in lower amounts in the dry powder, highlighting the possibility that some compounds may be more readily analyzed in the headspace of the liquid extract. Dry kava powder samples contained high levels of β-caryophyllene while the kava extract samples showed high levels of camphene. Both of these compounds are categorized as terpenes, which, along with terpenoids and hydrocarbons, were found in relatively high abundance among both types of samples.

Though these compounds have not been studied specifically in kava, many of them, such as camphene, α-pinene, β -pinene, and β-myrcene, are found in other species and have well-documented therapeutic effects across a number of broad classes of medical conditions. The increase in demand and continued consumption in both traditional and nontraditional contexts creates a strong need for further research into additional compounds found in kava beyond kavalactones and flavokawains in order to contribute to a more detailed understanding of the overall compound profile, and to correlate it with the broader range of therapeutic uses documented across the Pacific. A potential area of future research could involve investigating the chemical profile differences between various cultivars of kava and the physiological impacts associated with the chemical profiles. Differences between the compounds found in various kava cultivars creates the potential to consume specific cultivars based on desired effects or to target specific symptoms. Another aspect of continuing research could also involve the immersion of SPME Arrow into the kava extract, analyzing the liquid itself rather than the headspace, as well as investigating extractions with different temperatures and solvents from the kava itself.

Existing pharmaceuticals used for analgesia have potential side effects, some create dependencies, and having more options for analgesics would be a valuable benefit. Naturally occurring terpenes are an attractive group of compounds to employ for their pharmacological characteristics, and their widespread distribution across numerous readily available plants offers access to medicines to those who otherwise may not be able to acquire pharmaceuticals. Terpenes display a good therapeutic index: high bioavailability and acceptability, low toxicity and mutagenicity (some in fact, display antimutagenic properties), low side effect profile, and therapeutic doses significantly lower than doses shown to induce toxicity (Nuutinen, 2018). Investigating the traditional medicinal use of kava could provide a readily accessible alternative to existing pharmaceuticals.

Author Contribution

Cynthia Cheung: Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration **Jonathan Baker:** Conceptualization, Methodology, Resources, Writing – Original Draft, Writing – Review & Editing **Julianne Byrne:** Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization **Katelynn Perrault:** Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant Number 1752607, and the Howard Hughes Medical Institute 'Inclusive Excellence' program (Award 051316; Dr. David Asai, Dr. Patricia Soochan, Dr. John Matsui, Dr. Tykeia Robinson). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or HHMI. The project described was supported by grant number U54GM138062 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH), and its contents are solely the responsibility of the author and do not necessarily represent the official view of NIGMS or NIH. This work was also assisted with technical support by Thermo Scientific and SepSolve Analytical. The authors wish to thank Hilary Corcoran from Chaminade University of Honolulu for technical and maintenance support throughout the project duration. The authors also acknowledge the undergraduate research students within Instrumental Analysis classes occurring during Spring and Fall 2019 semesters at Chaminade University of Honolulu for their assistance in running kava samples on the instrument.

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