

# **Certificate of Analysis**

Sample Name: Bir Kar Instant

**Description:** Dehydrated aqueous kava root extract

 Process/Mix Date:
 23/09/2024

 Micro Lab Sampling Date:
 24/09/2024

 ASE Processing Date:
 27/09/2024

 UHPLC Injection Date:
 27/09/2024

 Date of Report:
 25/11/2024

Batch Number & Sample ID: B1SK

Analysis Identification Code: 240927U14

Plant Morphologist: Joses Laau
Chromatographer: John McGowan
Microbiologist: Ariane Urriza
Sample Preparation Technician: Eva David Livo
Quality Control Officer: Anabel Belen
Quality Assurance Manager: Dianne Manley

## **Executive Summary**

Total extracted major kavalactones = 7.10% of sample mass (w/w)

Chemotype = **423165** Moisture Content: **2.58%** (w/w)

Contamination = **Pass** Categorisation = **Noble kava** 

#### K and DHK Ratios

K to DHK: 1.40 DHK to K: 0.71
K to Y: 1.59 DHK to Y: 1.13
K to DMY: 3.17 DHK to DMY: 2.26
K to M: 4.13 DHK to M: 2.94
K to DHM: 4.14 DHK to DHM: 2.95

#### **Chemical Analysis:**

Sample Preparation: 1.000 g processed kava powder dispersed with silica sand to fill 10 mL Dionex™ ASE™ cell

Extraction method: Accelerated Solvent Extraction (ASE)

Extraction Process Automation: Thermo Scientific Dionex™ ASE™ 350 Accelerated Solvent Extractor

ASE Conditions – Solvent: HPLC grade Acetonitrile (ACN), Temperature: 60 °C, Pressure: 105 Bar, Pre-incubation: 5 min,

Static Hold: 20 min, Rinse Volume: 150%, Dilution to Working Range: 1 part ASE™ filtrate + 9 parts solvent (ACN) to give 1/10

**Pre-UHPLC Particulate Exclusion**: ASE  $^{\text{TM}}$  filtrate passed through Dionex  $^{\text{TM}}$  D28 cellulose filter prior to dilution to working concentration, then passed through 0.22  $\mu$ m hydrophilic PTFE filter prior to injection

### **Chromatographic Conditions:**

System: Thermo Scientific Vanquish Horizon Ultra-High-Performance Liquid-Chromatography

Instrument Components: VF-A10-A Split Sampler, VF-P10-A binary pump, VH-C10-A Column Compartment, and VF-D11-A Diode Array

Detecto

**Column:** 200 x 2.1 mm Hypersil GOLD, 1.9 μm particle size

Mobile Phase:5% isopropanol to 97% isopropanol in water (nonlinear). Total runtime 15.9 minutesColumn Temperature:60 °C, with active pre-heatingInjection Volume: 2.00 μLOrganic Modifier: None

**UV Detection:** 362 nm (aflatoxin B<sub>1</sub> and B<sub>2</sub> identification), 341 nm (flavokavain and aflatoxin G<sub>1</sub> and G<sub>2</sub> identification), 246 nm

(kavalactone identification), and 218 nm (kavalactone and aflatoxin secondary peaks); Peak identification assisted by elution time and spectrum matching. Relative quantification calculations based on channel 3

(246 nm)

Calibration Standards: Correlation coefficient for all identified compounds is greater than 99.995% on a 20-point calibration curve

derived by serial dilution of 1 ampoule of Cerilliant (kavalactones and flavokavains) and 1 ampoule of Ehrenstorfer (aflatoxins) certified analytical reference standards. Analytical balances calibrated with certified class OIML E2 weights with uncertainty +/- 0.000016 g (NATA accredited for compliance with ISO/IEC 17025,

by laboratory No.3279)

Instrument Method: Software: Chromeleon 7.2.10 Program: 240604 Hypersil iPrOH

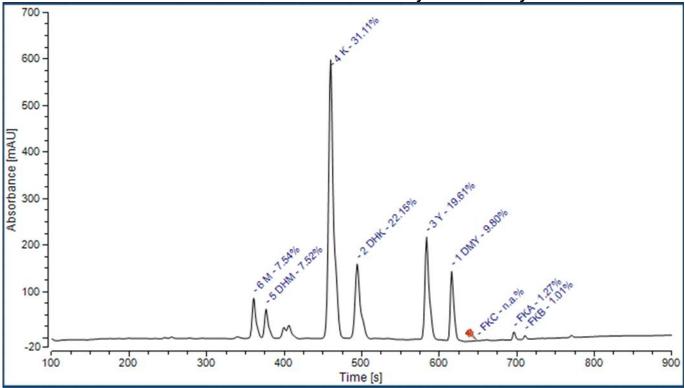
**Processing Method:** Software: Chromeleon 7.3.2 Program: 240606 Hypersil iPrOH Pro

**Confidence Probability:** Lower = 99.5% Upper = 99.5%

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# Determination of Relative Kavalactone Content by UHPLC Analysis at 246 nm



Peak labelled percentages represent that compound's abundance relative to the total amount of quantified compounds in the sample

Integration Results													
Chem #	Rel.Amt	Spectrum	Rtn T.	Rel.Ar	Rel.Ht	R^2	HV L <sub>0</sub> D	Cor.Coef	Cal.	Lower	Amount	Upper	Extracted
Name abv	%	Match	min	%	%	%	(mg/kg)	%	Pts.	Limit	(mg/kg)	Limit	% of mass
4 K	31.110	999.941	7.664	50.44	45.90	99.997	2.9065	99.998	20	22542	22615	22687	2.261
2 DHK	22.150	999.488	8.235	14.18	12.29	99.998	2.2489	99.999	20	16062	16102	16141	1.610
3 Y	19.611	999.589	9.726	15.72	16.89	99.999	2.0071	99.999	20	14225	14256	14288	1.426
1 DMY	9.800	999.007	10.266	8.88	11.42	99.998	2.2349	99.999	20	7100	7124	7148	0.712
6 M	7.536	999.254	6.010	5.54	6.62	99.998	2.3968	99.999	20	5453	5478	5503	0.548
5 DHM	7.516	994.844	6.277	4.07	4.82	99.998	2.0995	99.999	20	5442	5464	5486	0.546
FKA	1.268	997.492	11.602	0.78	1.38	99.994	0.4032	99.997	20	917	922	927	0.092
FKB	1.008	998.650	11.842	0.37	0.65	99.991	0.4956	99.996	20	728	733	738	0.073
FKC	n.a.	982.364	10.819	0.02	0.04	99.999	0.1742	99.999	20	n.a.	n.a.	n.a.	n.a.
Totals:	100%			100%	100%	Majo	r Kavala	ctones E	xtra	cted (%	of total	mass):	7.104
								A STATE OF THE RESIDENCE		1.10		and the same of the same	0.47

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-	lavokava	IDC -Vtr	actod (	rolativo	U/_ 1	. ) ) ( [
	lavunava	1115 L XU	acteur	leialive	/O I	2210

Flavokavains Extracted (% of total mass): 0.17

Peak Results												
Peak	Peak	Purity	Ret. T.	Signal	Peak to	Area	Height	Width	Туре	Res	Asym	Plates
Name	No.	Match	(S)	to Noise	Valley	mAU*min	mAU	50% min	1.00	(EP)	(EP)	(EP)
4 K	3	1000	459.864	36.4	924.31	75.568	598.526	0.17	BM *	3.26	1.39	31803
2 DHK	4	998	494.104	9.7	247.58	21.245	160.318	0.18	MB*	9.03	1.43	33787
3 Y	5	1000	583.544	235.6	n.a.	23.546	220.215	0.15	BMB	3.76	1.44	65655
1 DMY	6	999	615.944	632.2	n.a.	13.312	148.980	0.14	BMB	4.75	1.40	90427
6 M	1	999	360.584	5.2	62.82	8.299	86.318	0.14	BM	1.97	1.39	31431
5 DHM	2	997	376.604	3.8	45.71	6.099	62.807	0.14	MB	9.04	1.39	34117
FKA	8	995	696.104	16.0	47.69	1.174	17.963	0.10	BM	2.42	1.39	214879
FKB	9	974	710.504	7.6	22.53	0.559	8.486	0.10	MB	n.a.	1.23	228683
FKC	7	614	649.164	0.4	n.a.	0.028	0.471	0.04	BMB*^	7.94	0.82	197335

Disclaimer: The testing protocols employed utilise samples and are representative only of the respective batch, not necessarily other batches or products, even if apparently identical.

These analytical tests have been conducted by suitably qualified personnel on reputable equipment, using high-quality reagents and robust protocols, based upon industry standards. The results are generated in-house, and we believe them to be accurate and precise, however, despite our best efforts, errors may exist; No guarantee is expressed or implied.

These results should not be used as a final determination for use in a finished product; It is recommended that they be verified by the purchaser's quality control department and through the third-party services of an additional certified testing laboratory to ensure the purchased material meets specifications.

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## **Summary of Microbial Analysis:**

Microbial analyses are carried out in accordance with Forney Enterprises' Quality Assurance Programme. Analyses are performed in our modern, well-equipped, built-for-purpose microbiology laboratory, by experienced staff who are skilled in the art, using aseptic technique, calibrated equipment, and high-quality reagents, and incorporate the use of controls. Any given test may be performed using more than one method, substrate, or growth medium, including (but not limited to) 3M Petrifilm, HyServe Compact Dry plates, traditional and chromogenic agars, and culture-specific broths to validate results. We combine the use of industry standard protocols (such as FDA BAM and AOAC OMA) and proprietary methods developed in-house, however, the results reported are from the most sensitive method used (highest test counts). Forney kava is produced in a closely regulated HACCP certified facility, with continuous environmental monitoring and comprehensive testing throughout the production process. The figures below result from testing the finished product as packaged. Kava powder which does not meet the strictest criteria cannot bear the Forney logo.

Indicator Organism	Test Results (cfu/g)
Aerobic Plate Count (TPC)	90
Coliform	None detected
Escherichia coli	None detected
Yeast	None detected
Mould	None detected
Staphylococcus spp.	None detected
Salmonella spp.	None detected
Listeria spp.	None detected