ASSESSMENT OF KAVALACTONES IN KAVA, *PIPER METHYSTICUM* FORST. F., CULTIVARS OF PAPUA NEW GUINEA

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ABSTRACT

Assessment has been made of the kavalactones in eight cultivated varieties of kava, Piper methysticum, *grown in three locations in Papua New Guinea. A total of forty-seven samples of dried root, stem and peeling from Lae, Madang and the Keravat Germplasm Collection of the PNG National Agricultural Research Institute representing eight cultivars were analyzed by HPLC methods to determine the kavalactones. Three chemotypes are indicated, two identified by Lebot et al (1999) and another that is similar to that in* P. wichmannii. *Kavain, dihydrokavain and dihydromethysticin were found to be the predominate components. The Total KL (%) however is lower than found in kava from other South Pacific countries.*

Keywords: *Kava,* Piper methysticum, Piper wichmannii, *kavalactone, kavain, dihydrokavain, dihydromethysticin, methysticin, yangonin, desmethoxyyangonin.*

INTRODUCTION

Kava is a plant from the Piperaceae family and consists of two botanically differentiated species, *Piper wichmannii* C. DC. and *P. methysticum* Forst. f. The latter has been cultivated by people in Oceania for some 3000 years (Lebot 1997) and was first described in 1786 by a botanist traveling with Captain James Cook. Evidence has shown that *P. methysticum* is the sterile, cultivated form while *P. wichmannii* forms the fertile wild population (Lebot and Levesque 1996). New Guinea, Vanuatu and the Solomon Islands are thought to be the centers of origin of the kava plant, where the natural occurrence of *P. wichmannii* and other closely related species is isolated (Lebot *et al.* 1991).

Kava is a cash crop of several Pacific island nations. Kava die-back disease led exporters to search for alternative sources for production, cultivation and export. This led to an increased interest in kava cultivars grown in PNG. The PNG National Agricultural Research Institute (NARI), as the leading agency for research in agricultural crops, has produced information on the horticultural and agronomic aspects of kava cultivation. However, there had been limited information on the range of cultivars in PNG in terms of chemical components. As well as defining the chemical composition, an additional aim of the paper was to suggest the cause of strength of brew ('kick') reportedly found in the PNG Madang Short kava drink.

Kavalactones (a-pyrones and 5,6–substituted dihydro-a-pyrones) are psychoactive compounds released in a brew made by soaking the masticated or powdered root. The lactones are also present in the stump, stem, peelings and leaves. There are six major kavalactones each having different physiological effects. Studies into the pharmacology of these six kavalactones have been summarized by Hansel (1968) as narcotic (intensifying barbiturate narcosis), analgesic, local anaesthetic, contraction inhibiting and anticonvulsant, antispasmolytic and fungistatic towards select species of fungi and streptomyces. Kretzschmar's studies (1970; cited by Lebot *et al.* 1999) show the psychopharmacology of kavain is characterized by emotional and muscular relaxation, stabilization of the feelings and stimulation of the ability to think and act.

The work here is based on analysis of 47 samples that were received by the NARI Chemistry Laboratory from Keravat, Lae and Madang. Complete descriptions of the growth sites, soil conditions and morphotypes were not available at that time. This study is based on the observed chemotypic variation. Such data is essential for facilitating the selection of superior or commercially desirable cultivars (Lebot *et al.* 1991).

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MATERIALS AND METHODS

The samples were received in four accessions from Keravat (Lon.4° 20' N; Lat.152° 92' E), Lae (Lon.7° N Lat.146° 30' E) and Madang (Lon.6° 30'N; Lat.145° 30' E). The varieties were given local names: Madang Short (MS var.), Madang Tall (MT var.), Manus Green (ManG var.), Manus Pink (ManP var.), Manus Tall (ManT var.), Daru Tall (DT var.), West New Britain (WNB var.) and Kavieng (KAV var.). Samples were received as dry material but were further dried in a forced air draft oven at 105°C. The phytochemical analysis was made by High Performance Liquid Chromatography (HPLC) following the INA Method 101.002 first developed by Shao *et al.* (1998).

About 750 mg (±0.1mg) of finely ground stem, peeling, or root material was placed into a 50-ml volumetric flask along with 40 ml of methanol/ water (70/30) and sonicated for 60 minutes at room temperature. The flask was allowed to cool and contents diluted to volume with methanol/water (70/30). About 5ml was then filtered through 0.45mm nylon micro filters (Whatman) into a HPLC vial and capped. Kavain was used as the stock standard prepared as for the samples. The literature response factors were used to quantify the other five components. System suitability was ensured for the standards 0.01, 0.04, 0.10 and 1.00 mg/ml having a linear coefficient of 0.9999 and a resolution between desmethoxyyangonin and yangonin of 5.7. Analysis was carried out on a Varian Star Chromatography System Series 9000 consisting of a 9012 SDS, 9100 AutoSampler and a 9065 UV-Vis. A suitable analytical column, Microsorb MV, 5 mm (C-18), 4.6 ´ 250 mm substituted the reported YMCbasic 5 mm (C8). A temperature of 32-34°C was maintained in a water bath in which the column was immersed. Mobile Phase was isocratic acetonitrile: methanol: water: acetic acid (20: 20: 60: 0.1 v/v) at a flow rate of 1.0 ml/min. Injections of 10ml were made using a Valco valve and sample detection was made at 220 nm. Total run time was 45 minutes. Calculation of the components was made as follows:

$$\% \text{ Individual Kavalactone} = \frac{(A)(SR)(FV)(D)(F)(100)}{(W)}$$

Where: A = Peak area response of the kavalactone in the sample.

SR = The response of the corresponding kavalactone reference standard (slope of the calibration plot)

FV = The final volume of the sample preparation (ml).

D = The dilution factor of the sample preparation.

F = The correction factor for quantitation against kavain

W = The sample weight (mg).

HPLC analysis allowed separation of six major kavalactone (KL) components; in order of elution (reverse phase) they are: methysticin (M), dihydromethysticin (DHM), kavain (K), dihydrokavain (DHK), desmethoxyyangonin (DMY) and yangonin (Y). Chromatograph and retention times and response factors used to calculate the percent compositions are in Table 1. Dry extracts were not made as the method assays directly from solution.

Figure 1. **A sample chromatogram of kavalactones.**

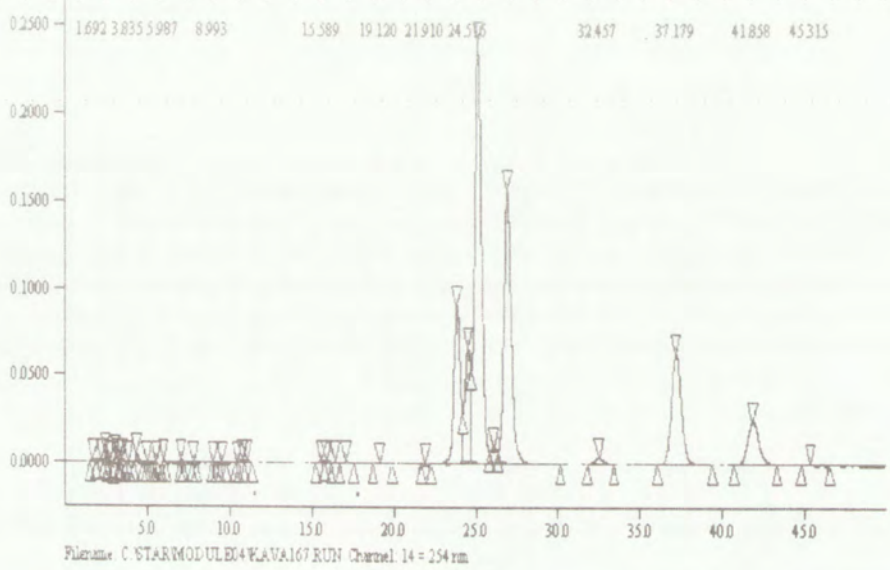

Table 1. **Retention Times for HPLC analysis above.**

Kavalactone	Retention Times (minutes)	Response Factors*
Methysticin (M)	23.847	0.6602
Dihydromethysticin (DHM)	24.516	1.638
Kavain (K)	25.117	1.000
Dihydrokavain (DHIL)	26.892	1.641
Desmethoxyyangonin (DMY)	37.179	0.9329
Yangonin (Y)	41.858	0.9669

* From INA Method 101.002 (Institute for Nutraceutical Advancement 2000)

RESULTS AND DISCUSSION

There were a total of 47 samples; 15 root, 20 stem and 8 peelings. An additional four samples were received in powdered form (labeled 'unknown'). The results of HPLC analysis and the chemical coding and chemotypes are presented in Table 2. Descriptive Statistics were applied to root and stem samples (Tables 3 and 4). Table 5 shows the results of simple linear correlation analysis for the roots and stems. Total Kavalactone (KL) percent variation in the 15 root and 20 stem samples was very large, coefficient of variance of 50.522 and 59.200 respectively. Desmethoxyyangonin had the greatest variance in both root and stem samples and yangonin had the least variance. High DMY content was observed in ManT and ManG and WNB varieties where they account for 43 – 60% of the total KL. Chemotypes of these three cultivars are characteristically low in dihydrokavain and yangonin. In Madang Short root, stem and peelings, kavain and dihydrokavain are almost equally expressed. In general, tested varieties showed higher levels of kavain, dihydrokavain and desmethoxyyangonin reflected in the chemotype codes. Lebot *et al.* (1999) found that environmental factors play an important role in the formation of kavalactones. Such factors include shading (negatively) and high levels of fertilization (positively). Similar correlations were not possible for this work. However, the samples originated from two distinct geographic locations. Lae and Madang are in mainland New Guinea and Keravat is in the Islands region. Keravat in East New Britain is known to have soil of volcanic origin whereas the Lae and Madang soil is alluvial. All locations have high rainfall (>2500mm annually) but wet periods are more regular in Lae and Madang. The plantings were well established in all three locations for five to ten years or longer. Madang samples had three of the highest total KL percent from the peelings. Madang sites grew only MS varieties but these showed two distinct chemotypes (codes: 245-163/613 and 425-163/613). Samples from Lae were of MT and MS. Lae kava displayed only chemotype C for both varieties grown, MS and MT. There were four samples of unknown origin and two of these were of a different chemotype (code: 425-163/613). Keravat germplasm consisted of all eight varieties and KL percent in these differed significantly at the 95% level. Keravat kava presented three chemotypes.

Overall there are three chemotypes observed here. Chemotype C: 245, 254 and F: 421, 425 have been assigned according to Lebot and Levesque (1989). Chemotype arbitrarily designated Q for codes: 142, 145, 146 are indicative of Vanuatu and PNG collections (Lebot and Levesque 1989). In Lebot *et al.* (1991), Table 1 of *P. wichmannii* and *P. methysticum* germplasms, lists PNG as having 23 wild forms, 4 cultivars and chemotypes; B (521634), C (254613), D (643251) and F (426315). Lebot *et al* (1999) found that KL percent 'decreases from the root to the stump, the basal stems and the leaves exhibit lower concentrations'. The group also found that 'peelings of the bark had a higher kavalactone content' (Lebot *et al.* 1999). The findings are similar for this work.

In general the appreciated effect [of kava brew] is correlated with high percentages of kavain and lower dihydromethysticin and dihydrokavain (Lebot *et al.* 1999). Chemotype F (426315) is the most appreciated by kava drinkers (Lebot 1990). PNG kava varieties showed high levels of kavain and dihydrokavain almost equally expressed and accounting for up to 78 percent of the Total KL percent Dihydromethysticin was also prominent and there were lower methysticin and yangonin. Kavain, DHK and DHM are of particular interest. Kavain and DHK both have local anaesthetic properties and DHM has a stronger narcotic effect (Hansel 1968). DHK has a relative analgesic effect stronger by dosage than that of acetylsalicylic acid (aspirin) (Bruggeman and Meyer 1963 in Hansel

1968). The predominance of these three kavalactones may be the underlying cause of the strength ('kick') appreciated by PNG kava consumers. PNG kava varieties however, showed lower Total KL percent content compared to that found in other kava producing countries.

Table 2. **Kavalactone HPLC analysis results of (47) Kava samples displaying chemotypes at three locations.**

Lab No.	Variety	Location	Section	Totals (%)	M	DHM	K	DHK	DMY	Y	Code	Chem otype
0143(1)	Madang short	Lae	stem	0.39	6.77	19.28	26.94	33.84	8.61	4.57	245163	C
0143(2)	Madang short	Lae	peelings	2.29	7.71	17.67	26.59	32.30	8.43	7.30	245163	C
0143(3)	Madang short	Lae	roots	6.55	7.10	11.30	31.47	34.50	10.75	4.88	245163	C
0143(4)	Madang short	Lae	peelings	4.69	7.36	13.64	29.68	34.02	9.74	5.57	245163	C
0143(5)	Madang short	Lae	stem	5.40	7.38	11.47	32.72	34.35	9.68	4.40	245163	C
0144(1)	Madang tall	Lae	stem	1.73	8.26	20.07	25.56	33.05	6.76	6.29	254613	C
0144(2)	Madang tall	Lae	stem	0.81	5.07	22.00	18.08	43.31	6.32	5.21	254136	C
0144(3)	Madang tall	Lae	peelings	3.54	8.84	19.07	26.05	30.27	7.33	8.44	245631	C
0144(4)	Madang tall	Lae	peelings	2.96	5.77	23.56	17.10	39.99	6.46	7.12	254316	C
0144(5)	Madang tall	Lae	roots	5.40	7.38	11.47	32.72	34.35	9.68	4.40	245163	C
0145	Madang	Lae	unknown	6.39	5.93	13.95	29.08	41.06	6.38	3.60	245163	C
0403	Madang short	Lae	unknown	3.74	8.33	11.26	37.71	26.33	8.57	7.79	425163	F
0507	Madang short	Lae	unknown	3.03	8.01	12.12	35.03	32.57	7.72	4.56	425613	F
0135 (a)	Madang short	Madang	roots	2.87	6.13	13.10	28.13	38.12	9.15	5.38	245163	C
0135 (b)	Madang short	Madang	stem	1.46	6.87	19.73	23.95	37.98	5.80	5.67	245613	C
0135 (c)	Madang short	Madang	peelings	2.45	6.93	20.11	23.43	35.28	7.11	7.14	245316	C
0139(1)	Madang short	Madang	roots	5.87	8.32	9.22	40.35	29.30	7.92	4.90	425613	F
0139(2)	Madang short	Madang	peelings	9.20	6.50	10.02	35.92	35.12	8.38	4.06	425163	F
0139(3)	Madang short	Madang	stem	2.79	8.08	14.21	34.48	33.22	5.45	4.57	425613	F
0139(4)	Madang short	Madang	stem	3.29	7.99	14.07	34.92	32.67	5.39	4.96	425613	F
0140(1)	Madang short	Madang	stem	2.98	7.50	13.83	34.04	34.26	5.75	4.63	245613	C
0140(2)	Madang short	Madang	peelings	10.47	6.30	9.80	36.21	34.88	8.63	4.19	425163	F
0140(3)	Madang short	Madang	stem	2.62	7.05	15.23	31.61	34.10	6.75	5.27	245613	C
0140(4)	Madang short	Madang	roots	4.30	8.38	9.40	39.79	27.75	8.61	6.08	425163	F
0141(1)	Madang short	Madang	peelings	11.88	6.58	11.06	33.30	35.85	8.28	4.94	245163	C
0141(2)	Madang short	Madang	stem	2.84	8.77	13.71	35.49	30.35	6.30	5.37	425613	F
0141(3)	Madang short	Madang	roots	3.59	9.41	12.68	36.37	30.01	6.52	5.01	425613	F
0141(4)	Madang short	Madang	stem	2.63	8.79	16.69	30.71	32.70	5.32	5.79	245631	C
0142(1)	Madang short	Madang	unknown	2.58	8.28	24.31	23.56	30.56	6.20	7.09	254631	C
0142(2)	Madang short	Madang	stem	3.22	8.84	22.10	23.13	31.50	6.21	8.22	245631	C
0142(3)	Madang short	Madang	roots	4.86	9.17	13.19	32.51	29.23	8.36	7.53	425613	F
3738	Madang short	Keravat	roots	3.86	7.94	9.81	38.00	27.82	10.36	6.06	421563	F
3739	Madang short	Keravat	stem	2.61	8.81	15.59	33.26	30.30	6.00	6.04	425631	F
3740	Manus pink	Keravat	roots	3.95	6.01	9.12	36.69	31.60	9.98	6.60	421536	F
3741	Manus pink	Keravat	stem	1.91	5.78	17.33	26.98	37.36	6.67	5.89	245136	C
3742	Manus tall	Keravat	stem	0.80	12.07	10.36	15.31	7.13	50.42	4.70	146523	Q
3743	Manus tall	Keravat	roots	0.48	11.18	10.84	12.30	7.92	52.38	5.38	146523	Q
3744	Manus green	Keravat	roots	0.64	11.92	8.10	10.59	4.76	60.28	4.36	164523	Q
3745	Manus green	Keravat	stem	0.56	11.45	12.13	21.74	7.13	43.01	4.54	145623	Q
3746	Kavieng	Keravat	roots	3.01	7.84	13.94	29.77	32.44	9.36	6.65	245163	C
3747	Kavieng	Keravat	stem	0.86	7.55	20.58	24.43	35.44	5.94	6.06	245631	C
3748	Madang tall	Keravat	roots	4.93	8.25	9.42	39.94	26.63	10.05	5.70	421563	F
3749	Madang tall	Keravat	stem	2.30	8.45	15.96	32.81	31.46	5.97	5.36	425613	F
3750	WNB	Keravat	roots	1.01	11.86	11.70	14.40	11.63	44.42	6.00	146523	Q
3751	WNB	Keravat	stem	0.72	10.60	12.13	22.16	14.95	34.49	5.67	142563	Q
3752	Daru tall	Keravat	roots	6.00	7.42	11.37	35.52	27.24	11.94	6.52	421563	F
3753	Daru tall	Keravat	stem	3.90	6.04	20.06	24.47	34.28	8.43	6.71	245136	C

Measured at 0.100AUFS

* Lebot et al, 1999 Chemotype coding.1 = DMY; 2 = DHK; 3 = Y; 4 = K; 5 = DHM; 6 = M. These are not the order of elution by RP- HPLC.

Table 3. **Descriptive Statistics Resulting from the analysis of 15 Root samples.**

	KL %	DMY (1)	DHK (2)	Y (3)	K (4)	DHM (5)	M (6)
Mean	3.822	17.982	26.220	5.696	30.570	10.979	8.554
Minimum	0.484	6.517	4.756	4.357	10.595	8.097	6.013
Maximum	6.551	60.276	38.115	7.534	40.348	13.942	11.918
Standard Deviation	1.931	18.085	9.969	0.909	10.104	1.758	1.859
Coeff. Of Variation	50.522	100.572	38.020	15.967	33.053	16.010	21.736

Table 4. **Descriptive Statistics Resulting from the analysis of 20 Stem samples.**

	KL %	DMY (1)	DHK (2)	Y (3)	K (4)	DHM (5)	M (6)
Mean	2.191	11.964	30.468	5.496	27.640	16.326	8.106
Minimum	0.385	5.316	7.126	4.400	15.310	10.365	5.071
Maximum	5.396	50.425	43.312	8.218	35.492	22.101	12.066
Standard Deviation	1.297	13.520	9.510	0.920	5.980	3.641	1.773
Coeff. Of Variation	59.200	113.00	31.213	16.749	21.634	22.304	21.873

Table 5. **Simple Linear Correlation analysis of Kavalactones in Root (15 samples) and Stem (20 samples).**

Roots Parameter				Stems Parameters			
X	Y	Corr. Coef.	Significance	X	Y	Corr. Coef.	Significance
DMY	DHK	-0.95	**	DMY	DHK	-0.94	**
	Y	-0.30	ns		Y	-0.29	ns
	K	-0.93	**		K	-0.60	**
	DHM	-0.30	ns		DHM	-0.57	**
	M	0.83	**		M	0.78	**
	Totals_%	-0.81	**		Totals_%	-0.46	*
DHK	Y	0.18	ns	DHK	Y	0.22	ns
	K	0.79	**		K	0.39	ns
	DHM	0.40	ns		DHM	0.65	**
	M	-0.91	**		M	-0.92	**
	Totals_%	0.75	**		Totals_%	0.36	ns
Y	K	0.25	ns	Y	K	-0.22	ns
	DHM	0.33	ns		DHM	0.65	**
	M	-0.18	ns		M	-0.11	ns
	Totals_%	0.09	ns		Totals_%	0.12	ns
K	DHM	-0.03	ns	K	DHM	-0.26	ns
	M	-0.73	**		M	-0.21	ns
	Totals_%	0.83	**		Totals_%	0.62	**
DHM	M	-0.15	ns	DHM	M	-0.63	**
	Totals_%	0.03	ns		Totals_%	-0.15	ns
M	Totals_%	-0.69	**	M	Totals_%	-0.25	ns

Table values are; Root, 0.6411 and 0.5139:Stem, 0.5614 and 0.4438; for 1% and 5% significance levels
** Significant at 1% and 5% level; ns, not significant

CONCLUSION

Kava, *P. methysticum* cultivated in Lae, Madang and Keravat today display three chemotypes C, F and Q. The last is likely to be a chemotype of wild forms from West New Britain and Manus. There are eight (8) cultivars that are locally identified in the Keravat germplasm collection. Of these cultivars Madang Short variety has been the most successfully produced and enjoyed kava brew. The major psychoactive components indicated for varieties having consumer preference are kavain, dihydrokavain and dihydromethisticin. Although the latter two compounds are considerably more potent, the lower Total (KL%) may be reducing their effect or there may be a modifying or synergetic effect with kavain, changing their physiological actions. This work presents a glimpse of the chemical variety of PNG kava that is now in cultivation but more work needs to be done to further the development of this crop.

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