



Analytical Methods

Detection of flavokavins (A, B, C) in cultivars of kava (*Piper methysticum*) using high performance thin layer chromatography (HPTLC)



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ABSTRACT

Kava (*Piper methysticum*) is used to prepare the traditional beverage of the Pacific islands. In Europe, kava has been suspected to cause hepatotoxicity with flavokavin B (FKB) considered as a possible factor. The present study describes an HPTLC protocol for rapid screening of samples. The objectives are: to detect the presence of flavokavins in extracts and to compare the FKB levels in different cultivars. Overall, 172 samples originating from four cultivars groups (noble, medicinal, two-days and *wichmannii*), were analysed. Results indicate that the ratio FKB/kavalactones is much higher in two-days (0.39) and *wichmannii* (0.32) compared to nobles (0.09) and medicinal cultivars (0.10). For each group, the ratios flavokavins/kavalactones do not change significantly between roots, stumps or basal stems and among clones, indicating that they are genetically controlled. This protocol has good accuracy and is cost efficient for routine analysis. We discuss how it could be used for quality control.

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1. Introduction

Kava, the traditional beverage of the Pacific islands, is made by cold water extraction of the underground organs of the plant species *Piper methysticum* Forst. Kava is also used as raw material by western pharmaceutical and nutraceutical industries for the relaxing and anxiolytic properties of its active ingredients called kavalactones. The German health authority, BfArM, declared in 2001 that kava based preparations could cause hepatotoxic reactions (Russmann, Lauterburg, & Hebling, 2001) and these products were banned for trade in Germany, Great Britain and France (Campo et al., 2003). BfArM recognised, however, that it was not known whether kavalactones or other components were responsible for the adverse effects (BfArM., 2003).

In 1998, when exports from the South Pacific islands reached their highest level, kava was amongst the top-selling herbs in the U.S. It has been estimated that the ban on kava caused a damage of US\$ 1.2 billion to the European industry (Ernst, 2007). The present trade restrictions are also an economic disaster for the producers and traders in Fiji, Samoa, Tonga and Vanuatu but kava is still an important cash crop satisfying a growing local market. In the traditional beverage, the intake of kavalactones by far surpasses the daily amount ingested with German preparations (Teschke,

Genthner, & Wolff, 2009). Signs of liver toxicity would thus be expected to occur more frequently in the Pacific (Stevinson, Huntley, & Ernst, 2002), but there is no evidence to suggest that kava is toxic in this region (Grace, 2003; Mills & Steinhoff, 2003; WHO, 2007).

The chemical composition of kava is complex and not less than 30 molecules have been identified from the roots, including nineteen kavalactones, three dihydrochalcones (flavokavins), and eight minor compounds. The two major determinants of kava quality are the chemotype and the total kavalactone content. The chemotype is determined by the relative proportion of the six major kavalactones: methysticin (M), dihydromethysticin (DHM), kavain (K), dihydrokavain (DHK), yangonin (Y) and desmethoxygangonin (DMY) (Lebot & Lévesque, 1996). The chemotype is responsible for the quality of the physiological effect, while the total kavalactones content determines its intensity. The chemotype is largely dependent on the cultivar and the organ of the plant used (roots, stumps or basal stems). The total kavalactones content also varies according to the cultivar, the organ, the age of the plant and the environment (Siméoni & Lebot, 2002; Wang, Qu, Jun, Li, & Bittenbender, 2013). In Vanuatu, noble cultivars used for daily drinking have a chemotype rich in K and its fast absorption causes a sudden relaxing effect. Cultivars rich in DHK and DHM produce nausea and are locally known as two-days (Lebot & Lévesque, 1989).

Before the European ban, processing companies applied an extraction process involving acetone that extracts kavalactones

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and other compounds including flavokavins A, B and C. Flavokavin A may have anticarcinogenic properties because of its growth inhibitory effect on bladder tumour cells in a mice model (Zi & Simoneau, 2005). Flavokavin B (FKB) has been considered as a possible cause for hepatotoxicity (Jhoo et al., 2006; Zhou et al., 2010). *In vitro* studies have shown that FKB derived from roots of the cultivar *Isa* from Papua New Guinea is cytotoxic to human hepatoma HepG2 cells with, however, effects yet to be elucidated (Jhoo et al., 2006). FKB was also present in ethanolic extracts of roots of the two-days cultivar *Palisi* from Vanuatu with a ten-fold higher amount compared to the noble cultivar *Ava La'au* from Samoa (DiSilvestro, Zhang, & DiSilvestro, 2007). In dried roots of an unknown cultivar from Vanuatu, analysis by gas-chromatography–mass spectrometry (GC–MS) showed for FKB peak areas of 0.1% and 0.5% for aqueous and acetic extracts respectively (Xuan et al., 2008).

In Vanuatu, the Kava Act has been passed into Parliament in December 2002 to regulate the use of the most suitable cultivars (Vanuatu Legislation., 2002). This law recognises 245 cultivars but only the 28 noble cultivars are authorised for export. The other 79 cultivars are considered as medicinal, 126 are classified as two-days and 12 as *wichmannii* and are unsuitable for daily consumption. It is, however, impossible to distinguish these cultivars when kava is chopped into sun dried pieces before export. Hence, quality standards are impossible to enforce without an efficient analytical method for the routine analysis of numerous samples (Teschke & Lebot, 2011). The 36th session of the *Codex Alimentarius* Commission noted the need to develop a regional standard and to address the gaps in the safety of the various products traded under the name “kava” (FAO/WHO, 2013).

Several protocols have been developed using gas chromatography (GC) or high performance liquid chromatography (HPLC) for the analysis and detection of kavalactones (Bilia, Scalise, Bergonzi, & Vincieri, 2004). Simultaneous HPLC-based detection of the six major kavalactones and the three flavokavins shows good accuracy (Meissmer & Haberlein, 2005). However, because HPLC is an expensive piece of equipment that makes use of important volumes of solvents, Near Infra-Red Spectroscopy (NIRS) has been tested for routine analysis of numerous samples. Calibration equations have been developed for major kavalactones and total kavalactones content analysed by HPLC (Gaub, Roeseler, Roos, & Kovar, 2004; Gautz, Kaufusi, Jackson, Bittenbender, & Tang, 2006; Lasme, Davrieux, Montet, & Lebot, 2008) and GC (Wang, Qu, Jun, Bittenbender, & Li, 2010) but flavokavins were not quantified.

Thin layer chromatography (TLC) has been used to isolate the six major kavalactones (Young, Hylin, Plucknett, Kawano, & Nakayama, 1966), to compare extracts (Loew & Franz, 2003), to concentrate the kavalactones and to reduce the amount of polymers (Schäfer & Winterhalter, 2005). Densitometry analysis was efficient to quantify kavain on silica gel plates but good results were obtained only when the solvent front attained a height of 18 cm after 3 h of developing time (Janeczko, Krzek, Pilewski, & Walusiak, 2001). Hence, the technique is difficult to use for routine analysis. High performance thin layer chromatography (HPTLC) is now commonly used to screen numerous samples on a single plate for the qualitative and quantitative analysis of secondary metabolites in plant extracts (Boudesocque, Dorat, Pothier, Gueiffier, & Enguehard-Gueiffier, 2013).

In the present paper, we describe an HPTLC protocol for the rapid screening of samples from different cultivars of kava. The objectives are: 1- to detect the presence of flavokavins in the extracts, 2- to compare the FK levels in different cultivars. We discuss how this protocol could be used for quality control and its practical implications for securing future markets for kava as a safe food.

2. Materials and methods

2.1. Kava cultivars

Root samples of 88 different accessions (corresponding to 72 distinct cultivars) were taken from 88 different plants grown in the germplasm collection of the Vanuatu Agricultural and Research and Technical Centre (VARTC) on Santo Island (Table 1). These accessions were planted together in a common plot and were all mature plants over 5 years of age; 22 were nobles, 10 were medicinal, 47 were two-days and 9 were *wichmannii* cultivars. Eight accessions (clones) of the noble cultivar *Borogu* and eight clones of the two-days cultivar *Palisi*, were also analysed to estimate intraclonal variation and confirm results between different plants. Roots, stumps, and basal stems were separated, washed, and cut into small cubic pieces of approximately 2 × 2 cm. Overall, 172 samples were prepared (88 roots, 42 stumps and 42 basal stems) and then dried at 60–80 °C until constant weight.

2.2. Samples preparation

Dry matter was ground in VARTC, Santo, using a Forplex F00 1218 hammer mill (Boulogne, France) into a kava powder with <2 mm particle size. Powder samples were then sent to Food Processing Lab (Department of Trade, Port-Vila, Vanuatu) and stored at room temperature before use. The samples were ground again using a kitchen mixer-grinder (MX-AC2105, Panasonic Corp., India) and then sieved with a 0.5 mm screen. The kava powder was weighed and dried for 24 h in an oven at 65 °C. 10 g of powder were extracted overnight in 30 mL of acetone and sonicated for 30 min before centrifugation at 4500 rpm for 10 min. The supernatant was then transferred to 5 mL vials and stored at 4 °C in the dark.

2.3. Standards

Methysticin, dihydromethysticin, kavain, dihydrokavain, yanguin and desmethoxyganguin were purchased from Sigma–Aldrich (Fluka) in France. Flavokavins A, B and C were obtained from Pr. H. Haberlein (Bonn, Germany). A second set of flavokavins were purchased from LKT Laboratories Inc. (St. Paul, USA). Standard stock solutions were prepared by dissolving the appropriate amount of each compound in acetone (1.0 mg/mL). Stock solutions were stored at 4 °C in the dark and were stable for several weeks. Peak purity tests were done by comparing UV spectra of the six individual kavalactones and three flavokavins in standard and sample tracks. For the determination of the linearity curve, different amounts of stock solutions (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 µL) of the six kavalactones and three flavokavins were applied on HPTLC plates that were developed and scanned at 254 and 366 nm. The calibration plots of peak areas versus concentrations were linear for all standards. Response factors were determined for each standard with R^2 coefficients all deemed acceptable above 0.99 (Table 2).

2.4. High-performance thin layer chromatography

Solvents (acetone, dioxane, hexane and methanol) were from Sigma–Aldrich. Analyses were performed with Merck (Darmstadt, Germany) silica gel 60 F₂₅₄ plates (20 × 10 cm), using a Camag (Muttentz, Switzerland) HPTLC system equipped with an automatic TLC sampler (ATS 4), an automatic developing chamber (ADC 2), a visualizer and a TLC scanner 4 controlled with winCATS software. Standards and sample solutions were applied band wise (band length of 8 mm, 250 nL/s delivery speed, track distance 8.0 mm

Table 1
List of accessions analysed by HPTLC for detection of flavokavins in kava roots.

Noble		Medicinal		Two-days		Wichmannii	
No.	Cultivar	No.	Cultivar	No.	Cultivar	No.	Cultivar
001	Borogu	013	Bagavia-1	002	Palisi	006	Sini bo
008	Puariki	025	Bagavia-2	003	Malogro	037	Maewo
012	Pade	045	Malogvelab	004	Tudei	040	Vambu
015	Kelai	161	Borogoru temit	010	Sentender	123	Mele liap
017	Nikawa pia	197	Borogu memea	011	Rongrongwul	155	Wichmanni
019	Ahouia	198	Borogoru memea	018	Fabukhai	155	Kau
041	Yevoet	203	Raimelomelo	020	Birfock	175	Maewo
042	Urukara	213	Tafandai	021	Mage	200	Malogu buara
064	Borogu	217	Maloglilab	022	Rogorogopula	207	Vambu
071	Borogu	218	Malogvelab	023	Tarivarus		
074	Borogu			024	Malovoke		
080	Borogu			030	Isa		
111	Seselaralara			031	Abogae		
113	Borogoru			034	Gelav		
115	Melomelo			043	Ranapapa		
121	Kerakra			066	Palisi		
137	Palarasul			068	Palisi		
138	Palasa			069	Palisi		
142	Poivota			075	Palisi		
160	Borogu			077	Palisi		
202	Borogu			103	Fabukhai		
212	Silese			104	Fabularalara		
				110	Sese		
				112	Tarivarusi		
				116	Taritamaewo		
				117	Tarivoravora		
				120	Gorogor entepal		
				122	Laklak		
				124	Tarivarus		
				126	Birfock		
				130	Palavoke		
				136	Makuku		
				139	Palisi long han		
				140	Palisi smol han		
				141	Pirimerei		
				145	Tudei		
				147	Vakorokoro		
				156	Isa		
				158	Abogae		
				159	Abogae tabal		
				171	Tarivarus		
				207	Raimelomelo		
				209	Nemleu		
				210	Poua		
				214	Ranapapa		
				215	Malogro smolhan		
				216	Malogro bighan		

Table 2
Ratios between flavokavins (FKA, B, C) and kavalactones (KL, Y = yangonin, DMY = desmethoxyyangonin).

	Roots					Stumps		Basal stems	
	No. acc.		FKA/Y + DMY	FKB/Y + DMY	FKC/Y + DMY	FK/KL	No. acc.	FK/KL	FK/KL
<i>Cultivars groups</i>									
Noble	<i>n</i> = 22	Mean	0.10	0.09	0.10	0.29	<i>n</i> = 8	0.26	0.27
		Sdv	0.04	0.04	0.04	0.10		0.08	0.07
Medicinal	<i>n</i> = 10	Mean	0.14	0.10	0.13	0.41	<i>n</i> = 4	0.32	0.30
		Sdv	0.03	0.05	0.03	0.08		0.08	0.10
Two-days	<i>n</i> = 47	Mean	0.39	0.39	0.24	1.02	<i>n</i> = 11	1.02	0.93
		Sdv	0.10	0.12	0.07	0.28		0.26	0.29
Wichmannii	<i>n</i> = 9	Mean	0.22	0.32	0.22	0.75	<i>n</i> = 3	0.71	0.74
		Sdv	0.06	0.05	0.05	0.15		0.18	0.17
<i>Clones</i>									
Borogu	<i>n</i> = 8	Mean	0.09	0.07	0.08	0.24	<i>n</i> = 8	0.23	0.27
		Sdv	0.04	0.03	0.03	0.08		0.05	0.06
Palisi	<i>n</i> = 8	Mean	0.39	0.38	0.26	1.03	<i>n</i> = 8	1.01	0.89
		Sdv	0.06	0.12	0.05	0.22		0.16	0.09

and distance from the edge of 15 mm). Plates were developed with hexane:dioxane (8:2 v/v) as the mobile phase (migration distance of 85 mm) at room temperature after 30 s plate predrying with 10 mL of mobile phase but no tank saturation. Visual inspection and documentation of the chromatograms were carried out at 254 nm and 366 nm. Plates were scanned under the following conditions: scanning mode, reflectance mode at 366 nm, D2 and W lamp slit dimension 8.00 mm × 0.20 mm, scanning speed 20 mm/s, data resolution 100 μm/step. Peak area measurement was used.

2.5. Statistical analyses

Statistical analyses were performed using XLStat software (Microsoft) for linearity curves, principal components analysis (PCA) and descriptive statistics on analysed samples.

3. Results

Pure standards applied on silica gel plates allowed the determination of R_f values for the nine molecules of interest (Fig. 1) as well as their UV spectra (Fig. 2). Accessions belonging to the two-days (track 1) and *wichmannii* (track 2) groups of cultivars displayed chromatograms clearly distinct from the noble cultivars (tracks 3–5). However, despite our efforts (tests with different plates, different mobile phases and development parameters), overlaps between K and Y and between DHM and K did not allow good separation of Gaussian type peaks at 254 nm. We could not obtain the complete separation of all six major kavalactones to confirm cultivars chemotypes according to the coding system previously developed with HPLC (Lebot & Lévesque, 1996).

Nevertheless, the observation of the plates at 366 nm allowed the clear visualisation of fully separated FKA, B and C. These molecules were easily visible in all two-days and *wichmannii* samples

while barely detectable in the nobles and medicinal samples. The heating of the plates at 105 °C for 5 min led to a sharpening of the bands and better visualisation. This technique was handy for plate image storage but was not used for compound identification. UV spectra (Fig. 2) indicate that FKA, B and C, as well as the two kavalactones (Y and DMY), have maximum absorbance at 366 nm while other substances (M, DHM, K and DHK) have very weak or nor absorption at this wavelength. Quantification of Y, DMY, FKA, FKB and FKC was therefore unaffected by other substances at 366 nm (Fig. 3) So that this wavelength was used for scanning plates with all samples corresponding to different accessions and cultivars (Table 1).

For the five pure standards scanned at 366 nm, the calibration plot of peak area versus concentration was polynomial. R^2 coefficients were very high for Y (0.9977, $Y = -5080.5x^2 + 17784x + 949.51$), DMY (0.9999, $Y = -33.325x^2 + 1023.7x + 196.28$), FKA (0.9986, $Y = -6371.5x^2 + 18248x + 192.53$), FKB (0.9996, $Y = -1964.8x^2 + 6264.1x + 139.46$) and FKC (0.9993, $Y = -3216.x^2 + 10984.x + 466.08$). Previous HPLC analyses have shown that yangonin (Y) is significantly correlated (at 1% level) with kavain (0.79), methysticin (0.55) and total kavalactones content (0.82) while desmethoxyyangonin (DMY) is significantly correlated with dihydromethysticin (0.60) (Lasmé et al., 2008). The sum of these two kavalactones (Y + DMY) is, therefore, assumed to be proportionate to the total kavalactones content in a sample. The results of the ratios between individual flavokavins (FKA, B, C) and the sum of yangonin (Y) and desmethoxyyangonin (DMY) are presented in Table 2 (FK/KL).

Overall, 140 kava extracts (88 roots, 26 stumps and 26 basal stems samples) were analysed by HPTLC. Multivariate analysis (PCA) of the data matrix of 88 root samples × 5 peak areas (FKC, Y, DMY, FKA, FKB) revealed that the three flavokavins are positively and significantly correlated (Pearson coefficient) to each other

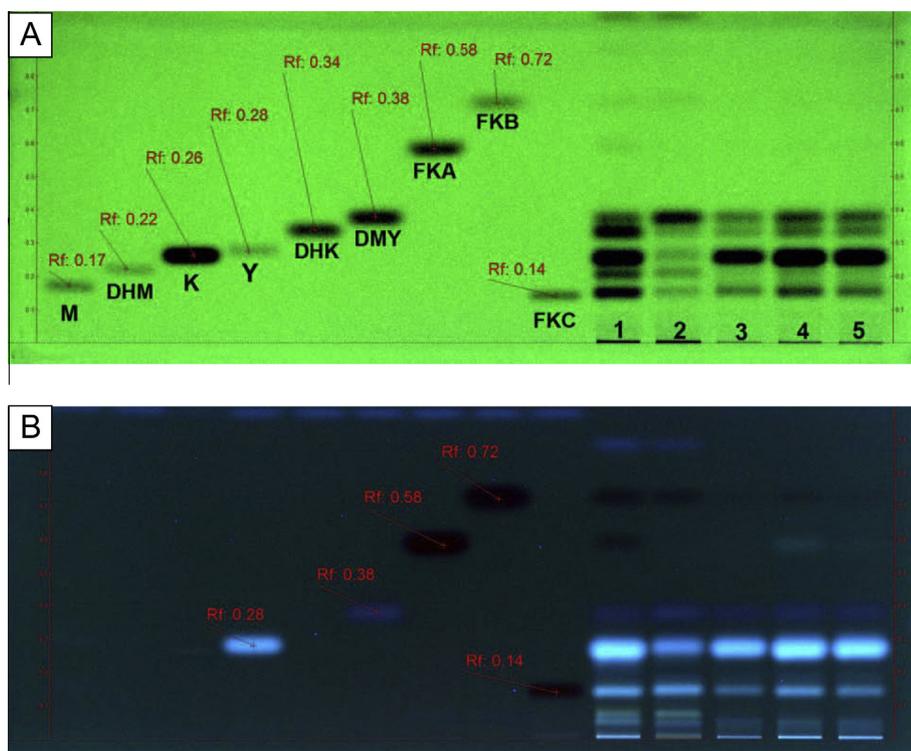


Fig. 1. (A) HPTLC Silica gel 60 F_{254} (20×10 cm plate) at 254 nm indicating the R_f values of the pure standards (M, DHM, K, Y, DHK, DMY, FKA, FKB, FKC). The chromatograms of kava samples correspond to: Track 1: two-days cultivar *Isa*, Track 2: *wichmannii* cultivar *Sini Bo*, Track 3: noble cultivar *Borogu*, Track 4: noble cultivar *Ni Kawa Pia*, Track 5: noble cultivar *Kelai*. (B) HPTLC Silica gel 60 F_{254} (20×10 cm plate) at 366 nm revealing the absorbance of Y (0.28), DMY (0.38), FKA (0.58), FKB (0.72) and FKC (0.14). Noble cultivars display low levels of flavokavins.

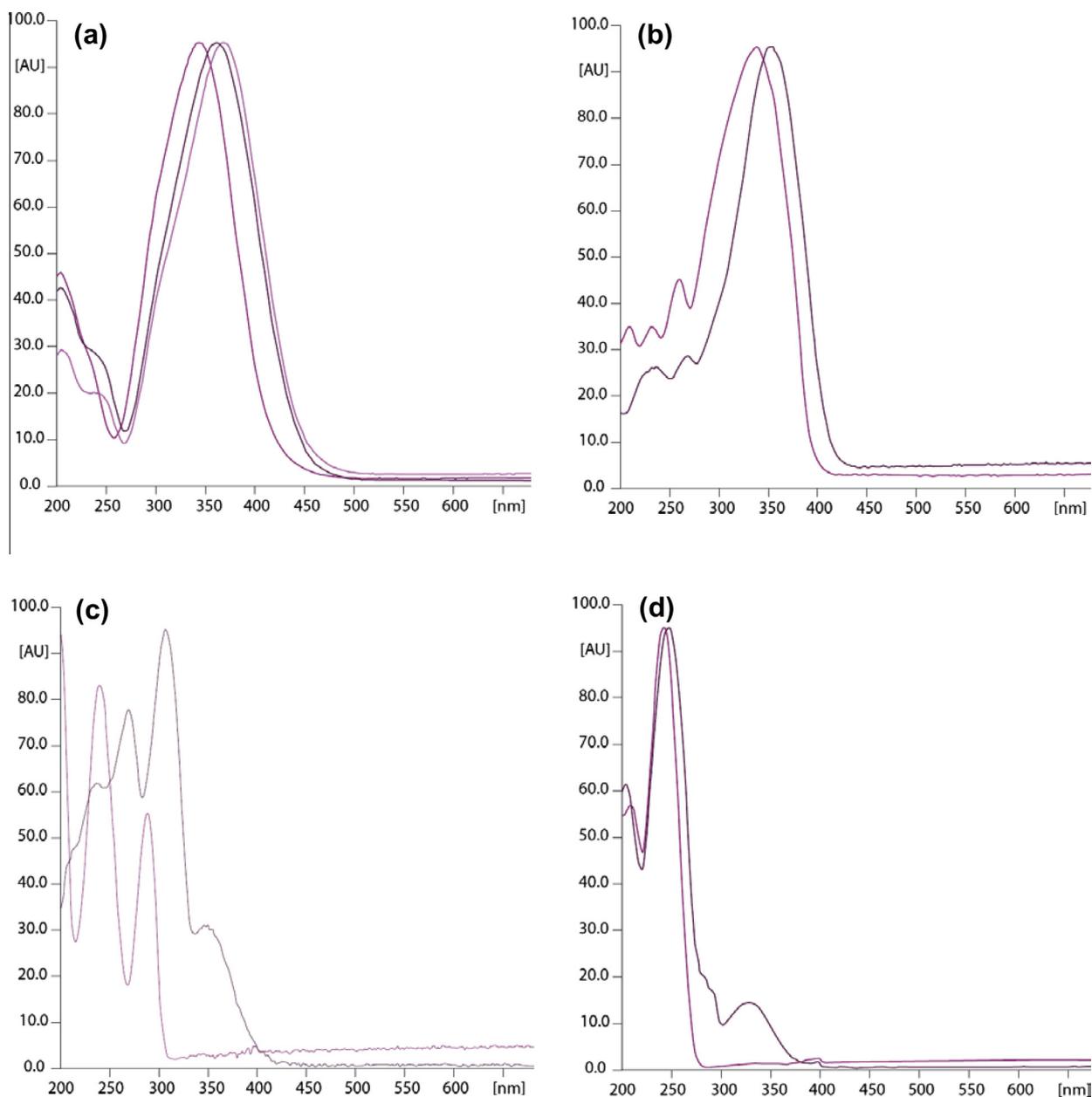


Fig. 2. (a) UV spectra of FKA, FKB and FKC (b) UV spectra of Y and DMY (c) UV spectra of M and DHM (d) UV spectra of K and DHK.

($FKA \times FKB = 0.955$, $FKA \times FKC = 0.942$, $FKB \times FKC = 0.939$) and so are the two kavalactones ($Y \times DMY = 0.677$). Results presented in Table 2 indicate that levels of $FKB/Y + DMY$ are much higher in two-days (0.39) and *wichmannii* (0.32) cultivars compared to nobles (0.09) and medicinal kavas (0.10). For each group of cultivars, the ratios FK/KL do not appear to change significantly when analysed in the stumps or the basal stems of the same accession.

The ratios are similar among the eight different clones of noble cultivar *Borogu* or two-days cultivar *Palisi* (Table 2). This indicates that these ratios (FK/KL) are controlled genetically. These results confirm previous studies conducted with HPLC on FKB. We have analysed cultivar *Isa* from Papua New Guinea and confirm results obtained by Jhoo et al. (2006). With a ratio FKB/KL of 0.41, the FKB content of *Isa* can be considered as very high. FKB was also detected in ethanolic extracts of roots of *Palisi* from Vanuatu with a ten-fold higher amount compared to the cultivar *Ava La'au* from Samoa (DiSilvestro et al., 2007). We could not analyse *Ava La'au*

but our HPTLC data indicate that *Palisi* has a FKB/KL ratio (0.38) more than five times higher than *Borogu* (0.07), a noble cultivar (Table 2).

4. Discussion

Like most cultivated tropical root crops, kava chemical composition is made of major compounds (starch, proteins, minerals, sugars, cellulose), useful secondary metabolites (kavalactones) and antinutritional factors. These toxic molecules are usually produced by the plant to protect itself against predators. They are usually down-selected during species domestication so that their levels are usually lower in domesticated crops than in their wild relatives. In the present case, it is obvious that flavokavins levels have been considerably diminished from *wichmannii* or two-days cultivars to medicinal and noble cultivars. The latter corresponding to

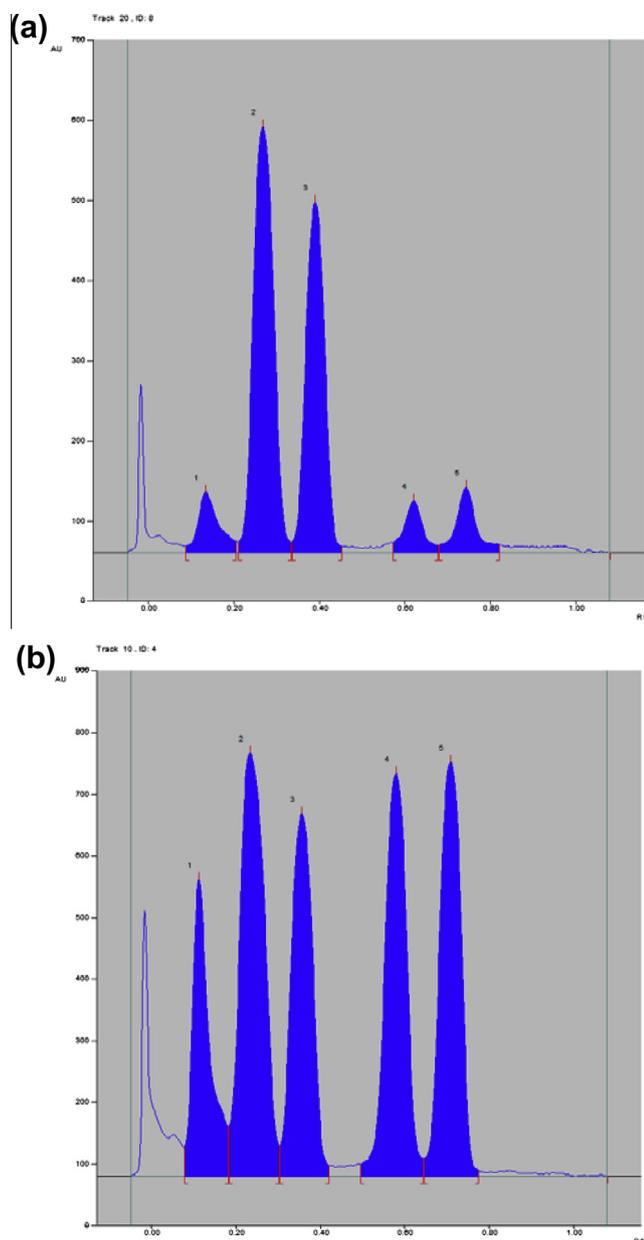


Fig. 3. (a) HPTLC chromatogram of the noble cultivar *Borogu*. From left to right, peaks correspond respectively to FK (1), Y (2), DMY (3), FKA (4) and FKB (5) scanned at 366 nm. (b) HPTLC chromatogram of the two-days cultivar *Palisi*. From left to right, peaks correspond respectively to FK (1), Y (2), DMY (3), FKA (4) and FKB (5) scanned at 366 nm.

the most improved cultivars suitable for daily drinking. In the case of kava, the determination of suitable qualities is reflected by the secular experience in the Pacific. In this region, experience tells that noble cultivars are safe and deliver the appropriate physiological effects with no hang-over. However, experience with two-days cultivars, indicates that they might possibly be connected with observations of liver toxicity. HPLC analyses (Siméoni & Lebot, 2002; Wang et al., 2013) have shown that noble cultivars have a high percentage of kavain over methysticin and dihydromethysticin. Our HPTLC results reveal that these suitable effects may also be due to the associated very low levels of flavokavins. Likewise, in HPLC studies the side effects of two-days cultivars were thought to be due to high levels of dihydromethysticin (Lebot & Lévesque, 1996). The present HPTLC results indicate that these side effects

could also be due to high levels of flavokavins. In other words, the detection of low levels of flavokavins is indirectly contributing to the identification of suitable chemotypes.

Before the ban in Europe, there was a lack of standardization of kava to be used as raw material for the pharmaceutical and nutraceutical industries, with no reference to molecules other than kavalactones in the extract. In Germany for example, quality specifications were developed a few years before the ban and were compiled by the regulatory agency but the requirements were rather succinct. The extracts ingredients had to be derived from peeled and dried stump so that the presence of toxins extracted from the bark could be avoided. Aerial parts such as stems, bark of the stumps exposed to light and leaves include an alkaloid known as pipermethystine (Dragull et al., 2003). It has been suggested that peelings may have been bought by European companies instead of the usual roots (SPC, 2001; WHO, 2007). Kavalactones were then extracted using organic solvents but alkaloids were most likely extracted too and so probably were flavokavins (Teschke et al., 2009). Lack of routine analyses might have caused problems to European companies. It has been suggested that flavokavins or alkaloids may be responsible for the cases of liver toxicity observed in Europe, provided this toxicity is accepted and established (Schmidt, 2003). Toxicological data on kavalactones does not allow attributing a potential health hazard to this fraction (Olsen, Frillo, & Skonberg, 2011).

Prior to the ban, the situation was similar in the Pacific for the traditional beverage and this represented a concern clearly expressed, as early as 2001, by the Secretariat of the Pacific Community in its producer's guide (SPC, 2001). In response to this concern, the Vanuatu parliament passed the Kava Act (Vanuatu Legislation, 2002) establishing that only noble cultivars should be traded, locally and for export markets. The rationale was that because these cultivars are consumed on a daily basis, it is likely that they do not present a danger for human health. Kava was not banned in the US where it is still sold as a dietary supplement. However, in 2002 the US Food and Drug Administration advised consumers on a potential hepatotoxicity (Teschke & Lebot, 2011).

In food and pharmaceutical sciences the use of marker substances for quality control is a somewhat frequent approach. The complete chemical composition of products is often quite complex and does not let itself to simple analysis for routine control. As FKB has been related to potential liver toxicity (Jhoo et al., 2006; Zhou et al., 2010), it is prudent to use it as a new marker for kava quality control, along with other flavokavins. Further investigation into these constituents is, however, warranted. FKB has also been reported to have potential usefulness for prevention and treatment of prostate cancer as well as significant anti-tumour effects on several carcinoma cell lines both *in vitro* and *in vivo* (Tang et al., 2010). Its efficacy profile suggests that FKB could be a promising novel chemotherapeutic agent (Ji et al., 2013). Until such examinations are available and complete, limit test for flavokavins may be advisable.

5. Conclusion

The present study demonstrates that the FK/KL ratio permits an unambiguous identification of noble kavas and exclusion of two-days kavas in exported material. We propose that this ratio could be used as an estimate of extract quality to guaranty the safety of kava. The HPTLC methodology described here offers a cost efficient technique (20 tracks per plate with only 10 mL of solvent) to screen numerous samples for rapid and efficient routine analysis of kava to be used for the preparation of the traditional beverage in the Pacific but also as raw material for western pharmaceutical and nutraceutical industries.

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