

# IMPACT OF KAVA CULTIVAR, PLANT PART AND EXTRACTION MEDIUM ON IN-VITRO CYTOTOXICITY OF KAVA (*PIPER METHYSTICUM*) IN HEPG2 AND HEP3B CELLS

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## Introduction

The MTT test as an easily applicable and validated tool for the detection of cytotoxicity of various compounds on liver cells of diverse species has been applied to kava preparations. The focus of our earlier work was, however, on regular kava qualities (Gebhardt and Schmidt 2005) – so-called “noble kava”, which have been used for ceremonial kava drinking in the South Pacific for centuries without any known problem. However, it is now known that in the past years unacceptable “kava” qualities (i.e. cultivars called “Tudei-kava” because of their two-day lasting effect causing hangover and adverse reactions) were used by some producers (Lebot 2006), with a coincidence in time with the first observation of liver case reports.

Since no toxicity was found to date with noble kava in vivo (Sorrentino et al. 2006; DiSilvestro et al. 2006) and in vitro (Gebhardt et al. 2005), regardless of the extraction solvent, our next aim was to screen for potential toxicity of Tudei-kava material deemed unsuitable for local use in the South Pacific, but used in European kava extracts.

Our interdisciplinary research program involved phytochemical analyses for kavalactone composition and pipermethystine content (Lechtenberg et al. 2006), and in vitro toxicity testing in human liver cells. Plant materials and extracts were selected to mimic marketed kava products as closely as possible, including the origin of the plant material.

## Materials and Methods

### Samples tested:

Two cultivars of *Piper methysticum* were selected:

- Ava La'au from Samoa, a cultivar favoured for daily drinking (“Noble kava”)
- Palisi from Vanuatu, a no-drink quality known to produce adverse effects such as headache or hangover (“Tudei kava”).

Both cultivars have been used for the production of certain German kava preparations.

### Plant parts tested

Roots vs. Peelings

### Extraction solvents used:

Acetone 75% vs. ethanol 96%

The analytical profiles of the kava extracts, obtained by micellar electrokinetic chromatography using an established method (Lechtenberg et al. 1999), are displayed in tables 1 and 2 (Nahrstedt et al. 2004).\*

Table 1: Analytical profiles of kava root extracts. Values in % of total kavalactones (KL)

	Ava La'au		Palisi	
	EtOH 96%	Acetone 75%	EtOH 96%	Acetone 75%
DMY	16.9	9.3	15.0	8.9
DHK	12.3	28.4	15.9	28.5
Y	16.5	12.5	13.7	11.8
K	29.8	21.2	28.1	22.1
DHM	7.8	15.7	10.7	15.4
M	16.7	12.8	16.4	13.3

Table 2: Analytical profiles of kava stem peeling extracts. Values in % of total kavalactones (KL)

	Ava La'au		Palisi	
	EtOH 96%	Acetone 75%	EtOH 96%	Acetone 75%
DMY	14.3	8.9	15.2	7.0
DHK	17.0	31.8	18.8	31.7
Y	14.2	12.4	13.5	10.5
K	25.1	16.7	27.9	17.4
DHM	11.4	20.3	11.0	20.9
M	18.0	12.0	15.6	12.6

### Assays:

- MTT assay (Gebhardt 1997)
- Rezasurin Blue Assay (Gaunitz and Heise 2004)
- Lactate dehydrogenase (LDH) leakage (Gaunitz and Heise 2004)
- Cellular ATP assay (Gaunitz and Heise 2004)
- Cellular GSH assay (Gebhardt and Fausel 1997)
- Morphological evaluation

## Results

No relevant toxicity was found for any of the tested materials and assays, which was confirmed by morphological evaluation of cell viability.

**Extracts from the roots of noble kava** were non-toxic in HepG2 cells, and showed cytotoxicity in Hep3B cells only in concentrations >> 3 mg/ml. No loss of intracellular ATP occurred below 3 mg/ml, with the exception of acetic extract, where ATP levels were affected at 500 µg/ml.

**Extracts from peelings of noble kava** were slightly more toxic than roots, but not in a relevant dose range (2.7-4 mg/ml). Loss of ATP occurred only above 2.5 mg/ml, with the exception of acetic extract, where ATP levels were affected at 500 µg/ml.

**Extracts from the roots of Tudei-kava** were more toxic than noble kava, but not in relevant dose ranges (2.4-3.5 mg/ml) by morphological evaluation; 1.25-3.6 mg/ml in the MTT and Rezasurin test. LDH leakage was observed at 2.5 mg/ml, with Hep3B cells reacting more sensitive to ethanolic extract, and HepG2 cells to acetic extract. A strong decrease of ATP was found in Hep3B cells with 0.4 mg/ml of ethanolic extract, and with 0.85 mg/ml of acetic extract.

**Extracts from peelings of Tudei-kava** were only slightly more toxic than the roots (2.1-5 mg/ml) by morphological evaluation, 0.8-5 mg/ml in the MTT and Rezasurin Blue assay). LDH leakage was observed with

2.5 mg/ml. Distinct loss of ATP occurred in Hep3B cells with 0.3 mg/ml of ethanolic extract, and 0.75 mg/ml of acetic extract.

Table 3: Results of toxicity testing in ethanolic extracts from Noble (N) and Tudei kava (T) roots (R) respectively peelings (P). Highlighted fields are results with borderline relevance.

	NR	NP	TR	TP
	Morphological evaluation (EC <sub>50</sub> , in µg/ml)			
HepG2	>>5,000	~3,500	~2,400	~2,500
Hep3B	>>5,000	~3,000	~3,000	~2,100
MTT test (EC <sub>50</sub> , in µg/ml)				
HepG2	>>5,000	3,500±100	1,300± 80	1,900±100
Hep3B	>5,000	3,000±100	2,000±100	1,350± 80
Rezasurin Blue Assay (EC <sub>50</sub> , in µg/ml)				
HepG2	>>5,000	3,200±100	2,900±110	2,900±120
Hep3B	>5,000	2,700±100	1,250± 80	800± 80
LDH leakage (fold control) at 2,500 µg/ml				
HepG2	0	0	0	0
Hep3B	0	0	3.0±0.6	3.1±0.5
ATP assay (EC <sub>50</sub> , in µg/ml)				
HepG2	>>5,000	2,700±100	2,000±250	950±50
Hep3B	3,000±160	2,500±100	400±70	300±50
GSH assay in HepG2 cells				
EC <sub>50</sub> (µg/ml)	>>5,000	3,750±120	1,850±100	1,850±90
%control	121	133	188	107

Table 3: Results of toxicity testing in acetic extracts from Noble (N) and Tudei kava (T) roots (R) respectively peelings (P). Highlighted fields are results with borderline relevance.

	NR	NP	TR	TP
	Morphological evaluation (EC <sub>50</sub> , in µg/ml)			
HepG2	>>5,000	~4,000	~2,900	>5,000
Hep3B	>5,000	~3,500	~3,500	~4,200
MTT test (EC <sub>50</sub> , in µg/ml)				
HepG2	~3,750	~3,500	~3,600	>5,000
Hep3B	~5,000	~3,000	~3,500	~3,700
Rezasurin Blue Assay (EC <sub>50</sub> , in µg/ml)				
HepG2	>5,000	~3,750	~3,000	>5,000
Hep3B	~5,000	~3,100	~2,900	~3,600
LDH leakage (fold control) at 2,500 µg/ml				
HepG2	0	0	3.0±0.3	2.4±0.5
Hep3B	0	0	0	0
ATP assay (EC <sub>50</sub> , in µg/ml)				
HepG2	>5,000	3,500±200	2,000±200	3,000±300
Hep3B	~500	~500	850± 90	750± 80
GSH assay in HepG2 cells				
EC <sub>50</sub> (µg/ml)	~3,750	~3,750	~1,850	~3,750
%control	107	117	220	112

Figure 1: Cytotoxicity of ethanolic extract from noble kava roots (MTT test)

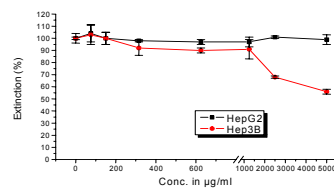


Figure 2: Effect of ethanolic extract from noble kava roots on LDH leakage

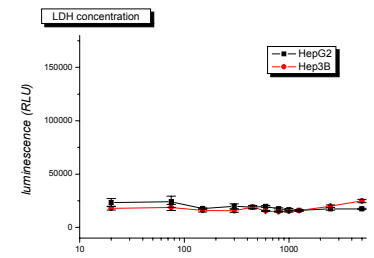
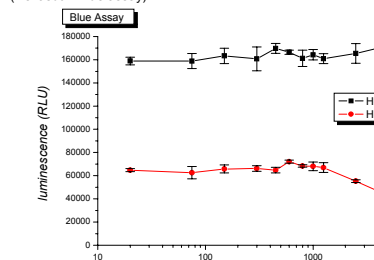


Figure 3: Cytotoxicity of ethanolic extract from noble kava roots (Rezasurin Blue assay)



## Discussion

No toxicity could be found for regular kava qualities (roots from noble kava). Otherwise the relative toxicity was as expected from experience in traditional use:

Noble kava, roots < Noble kava, peelings ≤ Tudei kava, roots = Tudei kava, peelings.

Figure 4: “False kava”, *Piper auritum*, is an example of a typical adulterant for kava which may degrade the quality of kava roots exported from the Pacific islands. More sophisticated, however, is the choice of unacceptable cultivars of “real” kava, *Piper methysticum*.



In all test models used the test results were far above relevant dose ranges, with the exception of some borderline results for peelings from Tudei kava and ATP assays with acetic extracts from noble kava.

## Conclusions

No liver cell toxicity in a relevant dosage range could be detected for standardized ethanolic and acetic kava extracts prepared from cultivars acceptable for daily kava drinking in the South Pacific. However, the possibility of toxicity induced by chronic ingestion of Tudei kava still needs to be addressed in vivo.

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