Anticancer activities of constituents of kava (*Piper methysticum*)

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ABSTRACT

Crude extracts of kava (Piper methysticum G. Forster, Piperaceae) showed good activity against ovarian tumour and leukaemia cancer cell lines. Bioassay-guided isolation resulted in the isolation of six known kava lactones and two flavokavains. The structure of the compounds were elucidated by spectroscopic techniques and by comparison with data in the literature.

Keywords: *Kava, Piper methysticum, Piperaceae, Lactones, Toxicity, Bioassay, Ovarian tumour, Leukaemia, NMR..*

1 INTRODUCTION

Kava (*Piper methysticum* G. Forster, Piperaceae) has been cultivated in the Oceania islands of the South Pacific for over 3,000 years (Hocart *et al.* 1993), and has a long history of being used as a remedy for the treatment of gonorrhoea, rheumatism, bronchitis, asthma as well as stomach aches and headaches (Weiner and White 1976). The kava lactones are believed to be responsible for biological activity which include local anaesthetic properties (Meyer and May 1964), sedative (Klohs *et al*. 1959; Meyer 1962), analgesic (Bruggemann and Meyer 1963), anticonvulsive (Meyer 1964; Meyer and Meyerburg 1964; Meyer 1965), antispasmodic (Meyer 1965), antimycotic (Hansel *et al.* 1966), anxiolytic (Pittler and Ernst 2000; Scherer 1998; Volz and Kiesser 1997), and central muscular relaxing effects (Baum *et al*. 1998. This has lead to its popular use in Europe and North America for the treatment of anxiety disorders (Singh and Blumenthal 1997). Some nineteen lactones have been isolated from kava with 6 major and 13 minor constituents (Shao *et al.* 1998) Also known to be present in trace amounts are the alkaloids: pipermethysticine (Smith 1979), *N*-cinnamoylpyrrolidine and *N*-methoxycinnamoylpyrrolidine (Achenbach and Wittman 1970), 3α,4α-epoxy-5β-pipermethystin and awaine (Dragull *et al*. 2003). Also known to be present are three flavokavains, flavokavain A-C (Achenbach and Wittman 1970; Dutta *et al*. 1973). The study (Steiner 2000) linking the low occurrences of some forms of cancer with kava drinking prompted us into this investigation. A screen of a crude extract of kava had shown good activity against ovarian and leukaemia cell lines. A bioassay-guided isolation on this extract lead to the isolation of six known kavalactones and two flavokavains. The paper describes the isolation, identification and the anticancer activities of the compounds.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

A kava plant was collected from the village of *Navakasali*, on the island of *Vanua Levu*, Fiji Islands. The plant was identified at the South Pacific Regional Herbarium at the University of the South Pacific. The samples were cleaned thoroughly in fresh water, cut into smaller pieces and sun dried. The dried samples were pounded to powdered form and stored in a plastic bag, sealed and sent by mail to the University of Aberdeen, Scotland where it was stored in a freezer at –20 ºC until extracted.

2.2 GENERAL EXPERIMENTAL PROCEDURES

Low resolution electrospray ionization mass spectra (LREIMS) were obtained on a Finnigan Masslab Navigator and high resolution mass data (HREIMS) were obtained on a Finnigan MAT-95. ^{1}H, ^{13}C and all NMR 2D experiments were recorded on a Varian Unity INOVA 400 MHz spectrometer, in $CDCl_3$ solution. Chemical shifts are reported in parts per million (δ) downfield relative to residual $CHCl_3$ at 7.27 ppm. HPLC separations were carried out using a Spectra Physics P100 isocratic pump and a Waters reversed phase (ODS, 250 x 10 mm) column and monitored using a Hewlett Packard HP 1050 Series Variable Wavelength UV Detector. UV and IR were taken on a Perkin Elmer Lambda 15 UV/VIS spectrophotometer and Ati Mattson Genesis Series FTIR machine respectively.

2.3 CHEMICALS

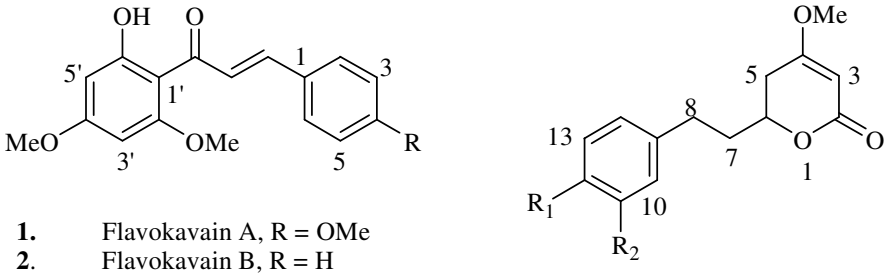
All extraction and partitioning solvents such as methanol, dichloromethane, hexane, ethyl acetate and *s*-butanol were laboratory grade (Sigma Aldrich). All HPLC solvents were HPLC grade (Rathburn Chemicals Ltd). Size exclusion column chromatography was performed on a Sephadex LH-20 (Sigma Aldrich) column. Flash column chromatography was performed on a Flash 40i system (Flash 40S, 32-63µm, 60Å cartridge). Normal and reversed phase TLC were carried out on silica gel and C-18 pre-coated plates (Merck) respectively.

2.4 EXTRACTION AND ISOLATION

Traditional preparation of kava involves extraction with water. The closest solvent to this is MeOH, which should extract most of the polar compounds. The use of dichloromethane was to improve the extraction efficiency of all polar as well as semi-polar compounds. The extraction was performed as follows: The powdered kava (500g dry weight) was extracted with MeOH (3x) and CH_2Cl_2 (3x), the solvent was removed under reduced pressure, and the extracts were combined. The crude oil was partitioned between water and CH_2Cl_2. The aqueous layer was then extracted with *s*-BuOH to give a yellow coloured oil (WB). The solvent was removed from the CH_2Cl_2 layer and the resulting oil was partitioned between

n-hexane and 10% aqueous MeOH. The *n*-hexane fraction was dried to give a brownish-yellow coloured oil (FH).

The MeOH layer was then phase adjusted to 50% aqueous MeOH and extracted with CH_2Cl_2 to give a dark brownish oil (FD). The 50% aqueous MeOH fraction was dried to give a light brownish coloured oil (FM). Interest was focused on the dichloromethane (FD) and hexane (FH) fractions, which were found to be highly active against ovarian tumour and leukaemia cancer cells. In addition, these fractions possessed interesting high and low field ^{1}H and ^{13}C NMR resonances. The FH fraction was subjected to normal phase flash chromatography using a mixture of hexane and ethyl acetate (80/20) as solvent, yielding 27 fractions. The column was eluted further by ethyl acetate (F-EtOAc) and finally methanol (F-MeOH).



1. Flavokavain A, R = OMe
2. Flavokavain B, R = H

Figure 1. Structure of kava flavokavains and lactones.

No.	Compound	R_1	R_2	C5-C6	C7-C8
3.	dihydromethysticin	O-CH_2-O		—	—
4.	7,8 dihydrokavain	H	H	—	—
5.	kavain	H	H	—	==
6.	demethoxyyangonin	H	H	==	==
7.	*cis*-yangonin	OMe	H	==	==
8.	*trans*-yangonin	OMe	H	==	==

After monitoring by TLC, similar fractions were pooled together and then purified by HPLC. The fraction (F12-18) was purified by normal phase HPLC using a mixture of hexane and ethyl acetate (80/20) to afford 14.2 mg of flavokavain A (**1**) while the fraction F6-11 yielded 8.2 mg of flavokavain B (**2**). The fraction (F-EtOAc) was purified by reversed phase C-18 HPLC using a mixture of water, methanol and acetic acid (70/30/0.1) as solvent to yield 6.3 mg of dihydromethysticin (**3**) and 8.4 mg of 7,8-dihydrokavain (**4**). The FD fraction was purified as above to afford 21.6 mg of kavain (**5**), 11.6 mg of demethoxyyangonin (**6**), 9.3 mg of compound of *cis*-yangonin (**7**) and 8.4 mg of *trans*-yangonin (**8**).

Table 1. Anticancer activities of kava crude fractions and isolated compounds.

Sample	K562 (µg/mL) leukaemia	A2780 ovarian tumour
WB	>100	48.16
FD	0.85	0.43
FH	0.70	0.66
flavokavain A (**1**)	2.04	1.32
flavokavain B (**2**)	0.95	0.56
dihydromethysticin (**3**)	6.43	5.15
7,8-dihydrokavain (**4**)	9.15	4.87
kavain (**5**)	5.35	2.54
demethoxy-yagonin (**6**)	2.88	3.79
cis-yagonin (**7**)	0.42	0.75
trans-yagonin (**8**)	1.41	2.39

2.5 MTT ASSAY

The anti-tumour assay was performed at the Paterson Institute for Cancer Research, at the Christie Hospital in Manchester, UK. Tests were performed using leukaemia and ovarian tumour cell lines. The K562 human leukaemia and the A2780 ovarian cell lines were cultured as described in the literature (McGowan and Fox 1988). Cytotoxicity tests were carried out using the MTT assay described in the literature (Mossmann 1983). Cells were treated with the drug in 96 well plates in antibiotic free RPMI medium containing 10% foetal calf serum. Drugs were dissolved in dimethylsulphoxide (DMSO) and were

added by serial dilution. Drug treatment lasted 5 days, the duration of the assay. The IC_{50} value was calculated by reference to a standard curve constructed for control cells.

3 RESULTS AND DISCUSSION

Structures of all the compounds were confirmed by interpretations of 1D, 2D NMR and HRESIMS data and by comparison with spectroscopic data in the literature (Dharmaratne *et al.* 2002). The anticancer activity of the eight compounds is shown in Table 1 with IC_{50} values ranging between 0.42-9.15 µg/mL. It is reasonable to suggest that the anticancer activity observed in the FD and FH crude fractions is due to the presence of *cis*-yagonin and flavokavain B. The relatively high activity of one of the isomeric forms of yagonin is interesting from a structure-activity perspective. *Cis*-yangonin was an order of magnitude more potent than its geometric isomer, as well as the compound demethoxyyangonin which was found by a previous study (Sotheeswaran *et al*. 2002) to inhibit the release of the tumour necrosis factor α (TNF α). The mode of activity of flavokavain A has been studied and is known to induce apoptosis in bladder cancer cells by involvement of Bax protein-dependent and mitochondria-dependent apoptotic pathway (Zi and Simonaeu 2005).

4 CONCLUSION

This study has substantiated the findings of previous studies about the anticancer activities of kava. The most active compounds against leukaemia and ovarian tumour are *cis*-yagonin and flavokavain B.

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