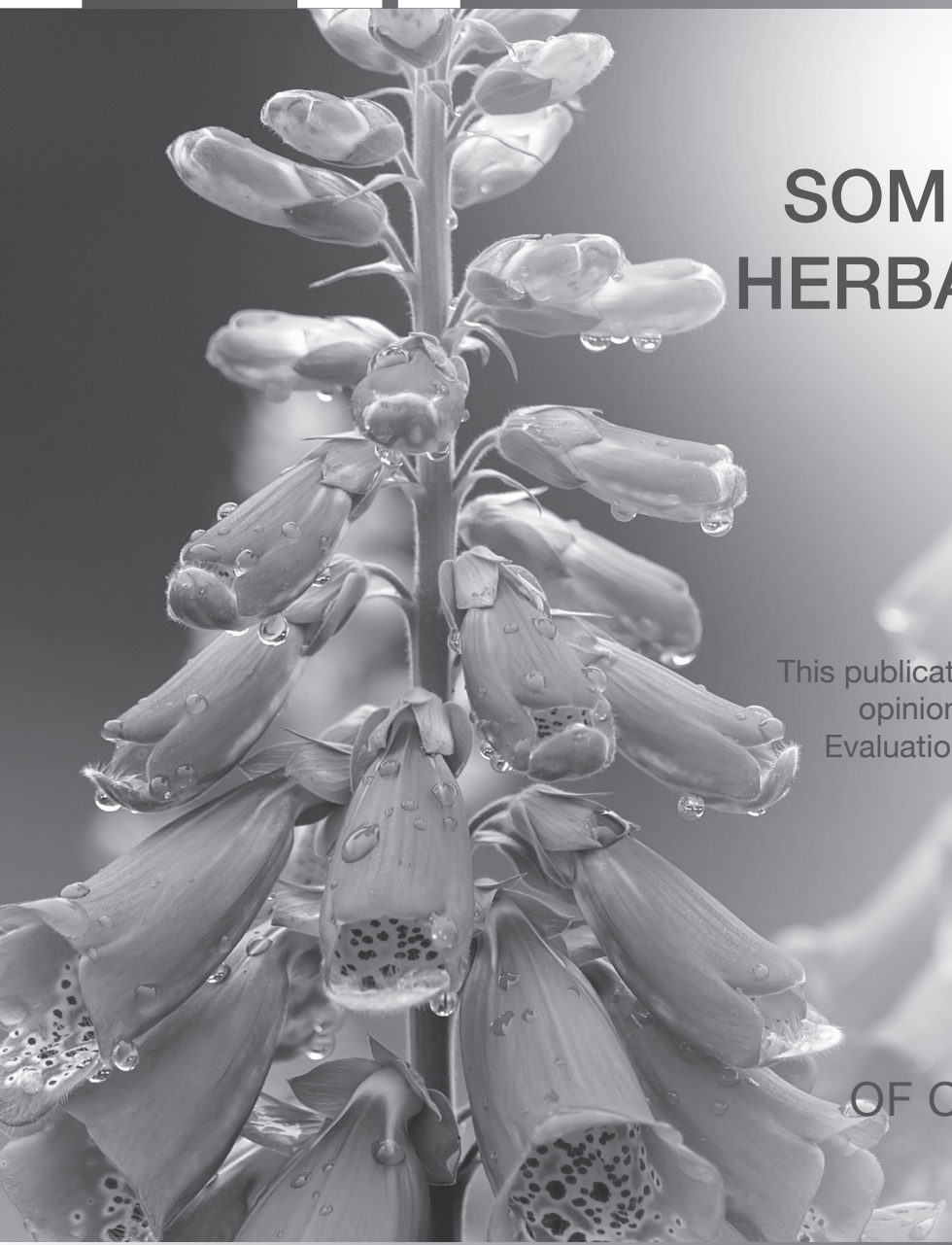
IARC MONOGRAPHS



SOME DRUGS AND HERBAL PRODUCTS

VOLUME 108

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

KAVA

1. Exposure Data

The kava plant is indigenous to Oceania (Lebot *et al.*, 1997; Ramzan & Tran, 2004) and has been used both ceremonially and recreationally in certain cultures of the South Pacific for at least 1500 years. Europeans documented its use when they travelled to Polynesia in the 18th century (WHO, 2007). The cultural history of the use of kava has been reviewed by Singh (1992).

In the past, traditional use of kava was widespread, but, in certain cultures, custom determined who could use kava and for what purposes. In recent years, as part of the processes of modernization, major changes have occurred with regard to who uses kava, and where and how it is consumed. In some places, kava is now being consumed much like alcohol in western countries, as a beverage that is drunk socially (McDonald & Jowitt, 2000).

1.1 Identification of the agent

1.1.1 Botanical data

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 9000-38-8
Chem. Abstr. Name: Kava-kava resin (8Cl)
Botanical name: *Piper methysticum* G. Forst
Family: *Piperaceae*
Genus: *Piper*
Plant part: Rhizome

(WHO, 2004; O'Neil *et al.*, 2006; NTP, 2012; and SciFinder, 2013)

According to WHO (2007), some other species such as *Piper wichmanni*, *Piper aduncum* and *Piper auritum* have also been marketed as "kava."

Common names: Kava; Kava-kava; Ava-ava; Antares; Ava; Ava pepper; Ava pepper shrub; Ava root; Awa; Fijian kava; Gea; Gi; Grog; Intoxicating long pepper; Intoxicating pepper; Kao; Kava kava rhizome; Kava root; Kavapiper; Kavapyrones; Kavarod; Kavasporal forte; Kavekave; Kawa; Kawa kawa; Kawa pepper; Kawa Pfeffer; Kew; Long pepper; *Macropiper latifolium*; Malohu; Maluk; Maori kava; Meruk; Milik; Pepe kava; Piperis methystici rhizome; Rhizoma piperis methystici; Sakaua; Sakau; Tonga; Yagona; Yangona; Yaqona; Yongona

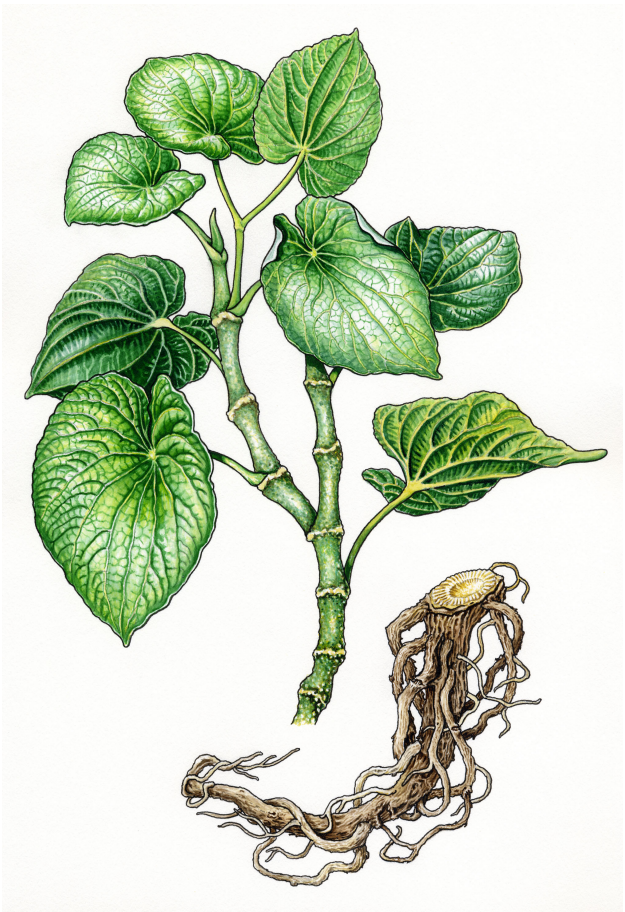
(b) Description

See Fig. 1.1

The tropical shrub *Piper methysticum* is a hardy, fairly succulent, slow-growing perennial that is widely cultivated in Oceania. The species is sterile and reproduces asexually. Due to its traditional use as a ritual beverage known for promoting relaxation and a sense of well-being, the kava plant spread widely throughout Oceania, in Polynesia, Melanesia, and the Federated States of Micronesia (Norton & Ruze, 1994; NTP, 2012).

The leaves are heart-shaped, pointed, 8–25 cm in length, and smooth and green on both sides. Kava is cultivated for its rootstock (rhizome), also

Fig. 1.1 *Piper methysticum* G. Forst



From Spohn (2013)

referred to as the stump. The stump is knotty, thick, and sometimes tuberous and often contains holes or cracks created by partial destruction of the parenchyma. A fringe of lateral roots up to 2–3 m in length extends from the pithy rhizome. The roots comprise a multitude of ligneous fibres and consist of > 60% starch. Rhizome colour varies from white to dark yellow, depending upon the amount of kavalactones contained in the lemon-yellow resin. The plant is usually harvested when it is about 2–2.5 m in height (Singh, 1992; Lebot *et al.*, 1997; NTP, 2012).

The cultivation and selection of kava has produced numerous varieties or cultivars recognized by differences in the internodes (space between stem joints), colour of stems, intensity of leaf colour, and quality of the root. Different varieties are classified, named, and used for different purposes by the indigenous people (NTP, 2012).

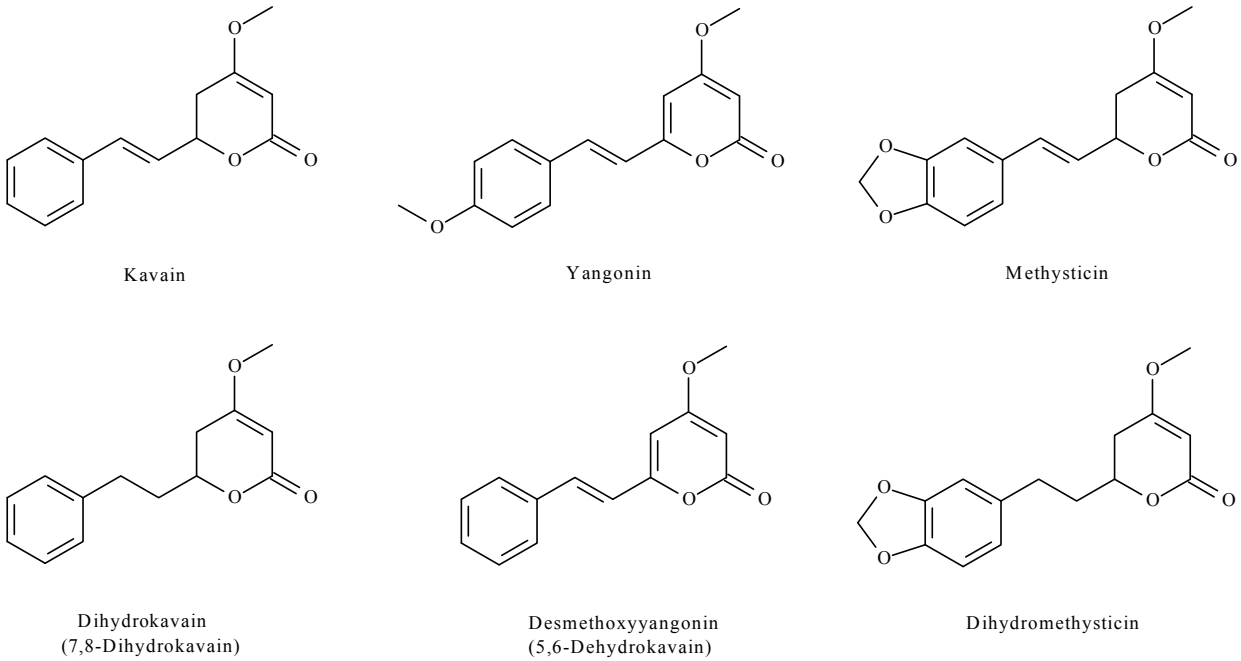
The dried rhizome consists of irregular, transverse and longitudinal pieces, varying considerably in size and shape: 3–20 cm in length and 1–5 cm in diameter. The outer surface is light yellowish or greyish-brown, longitudinally wrinkled, with large, whitish, circular root scars. The fracture is coarsely fibrous, the inner surface is yellow-white, with thin bark, radiate xylem, and large pith (WHO, 2004).

1.1.2 Chemical constituents and their properties

Analysis of the composition of kava rhizome indicates that the fresh material is on average 80% water. When dried, the rhizome consists of approximately 43% starch, 20% fibres, 12% water, 3.2% sugars, 3.6% proteins, 3.2% minerals, and 15% kavalactones, although the kavalactone component can vary between 3% and 20% of the dry weight of the rhizome, depending on the age of the plant and the cultivar. The bioactive principles of kava rhizome are mostly, if not entirely, contained in the lipid-soluble resin. The compounds of greatest pharmacological interest are the substituted α-pyrones or kavapyrones, commonly known as kavalactones. At least 15 lactones have been isolated from kava rhizome. The following six compounds are present in the highest concentrations and account for approximately 96% of the lipid resin: kavain, dihydrokavain, yangonin, desmethoxyyangonin, methysticin, and dihydromethysticin (see Fig. 1.2). Other constituents of kava include chalcones and other flavanones, and conjugated diene ketones (Shulgin, 1973; Dentali, 1997; WHO, 2004; NTP, 2012).

In the past, "kavain" has been used to indicate a racemic mixture resulting from chemical synthesis, and "kawain" for the naturally

Fig. 1.2 Structures of the major kavalactones occurring in kava rhizome



Compiled by the Working Group

occurring compound, which is a dextro-isomer. Currently, the two terms, kavain and kawain, are frequently used interchangeably in the scientific literature, but the term kavain has started to supersede kawain (Singh, 2004a).

The chemistry of kava and kavalactones has been reviewed in detail by Ramzan & Tran (2004).

1.1.3 Technical and commercial products

Kava biomass is normally sold as the rhizome, with the periderm and roots removed. The peeled rhizome is also the desired material for solvent extraction to produce kava extracts. Kava may also be sold as an unpeeled rhizome covered with the cork or with the roots attached. Peelings from the root and stump have also been used in commerce (Morgan *et al.*, 2005). Powdered forms of rhizome are available in commercial markets in Fiji and have been described to be adulterated to the extent that they only contain 71–78% of the expected active constituents (Clough *et al.*, 2000). Singh (2004a) mentioned adulteration with sawdust, flour, or soil. Adulteration of kava with plants resembling the genuine kava, but lacking the kavalactones and the distinct kava odour has also been reported. The main "false kava" species are *P. auritum* and *P. aduncum* (Singh, 2004b).

Very few data exist on kava contamination by bacteria or with mycotoxins (Teschke *et al.*, 2011). A study on ochratoxin A contamination found concentrations of 3.0 ng/g in one sample of kava root (Trucksess *et al.*, 2006). The level of contamination with aflatoxin B_1 in four samples of ground kava was 0.5 ng/g (Weaver & Trucksess, 2010).

The part of the plant used, processing techniques, and specifically the extraction solvent and the ratio between solvent/plant material in the case of kava extracts, may have considerable

influence on the chemical composition of the end product. For example, the alkaloid pipermethystine was not detectable in some commercial kava extracts (Teschke *et al.*, 2011).

[The Working Group noted that the influences on composition mentioned above may hinder the comparison of studies, especially if the applied kava material was not specified exactly.]

1.2 Analysis

The chemical analysis and quality control of both kava and its extracts obtained by aqueous acetone or aqueous methanol, and supercritical fluid extraction – typically with carbon dioxide modified with methanol as solvent – were reviewed by Bilia *et al.* (2004). Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) can be used for the analysis of kavalactones with some advantages and disadvantages for each method. Using GC analysis, methysticin and yangonin, which are two of the major components, are generally not separated. In addition, the high temperature of the injection port causes the decomposition of methysticin. Concerning HPLC analyses, reversed-phase separation is generally better because it is highly reproducible with a very low detection limit for all compounds even if the quantitative analysis of the kavalactones by HPLC needs to be carried out in the absence of light to prevent the *cis*/*trans* isomerization of yangonin (Bilia *et al.*, 2004). Besides various chromatographic approaches reviewed by Bilia *et al.* (2004), near infrared spectroscopy and nuclear magnetic resonance spectroscopy have been suggested to directly determine kavalactones without the need for separation (Table 1.1).

1.3 Use

1.3.1 Indications

(a) Medicinal use

According to WHO (2004), the medicinal uses supported by clinical data are short-term symptomatic treatment of mild states of anxiety or insomnia due to nervousness, stress or tension; the medicinal uses described in pharmacopoeias and in traditional systems of medicine are to induce relaxation, reduce weight, and treat fungal infections. Uses described in traditional medicine, but not supported by experimental or clinical data, are treatment of asthma, common cold, cystis, gonorrhoea, headaches, menstrual irregularities, urinary infections, and warts.

The German Commission E has approved kava for use in conditions of nervous anxiety, stress, and restlessness (Anonymous, 2000).

(b) Traditional food and recreational use

A local traditional drink, also known under the name kava, is obtained from the root or rhizome of the kava plant. The kava drink is made from water extracts, with water-insoluble substances made available to the drinker by emulsification, which may be accomplished by pounding or chewing of the rhizome (WHO, 2007).

On some islands in the South Pacific, fresh kava root or rhizome is used to prepare the traditional drink, while on others it is the dried and ground roots or rhizomes that are used. For fresh preparations, the root is chewed by young women, who spit the juice into the kava bowl without swallowing it themselves. The juice is then mixed with water or coconut milk and further processed. Most people drink only the water extracts of kava. This is obtained by adding water to kava roots which are finely ground and then filtered using cheese-cloth (WHO, 2007).

The kava drink has been described to have a psychoactive activity, and potency can vary

Table 1.1 Selected methods of analysis of constituents of kava in various matrices

Sample matrix	Analyte/purpose of analysis	Sample preparation	Assay method	Detection limit	Reference
Serum and urine	Kavain and metabolites/metabolism	Glucuronidase treatment, extraction with dichloromethane:diethylether (7:3, v:v)	HPLC-DAD and LC-MS	1 ng/mL	Tarbah *et al.* (2003)
Urine	Kavalactones/metabolism	Chloroform extraction	GC-MS, HPLC	NA	Duffield *et al.* (1989)
Kava root from botanical supplier	Ochratoxin A/contamination	Immunoaffinity column cleanup	HPLC-FD	NA	Trucksess *et al.* (2006)
Dried kava roots	Kavain and other major kavalactones/isolation	Ethanol extraction	HSCCC	NA	Schäfer & Winterhalter (2005)
Commercial kava extract, and kava finely powdered	Kavalactones and a range of other compounds/quality assessment	Solution with DMSO-d_6	NMR	NA	Bilia *et al.* (2002)
Kava root extract	Kavalactones/structural elucidation	Extraction with methylene chloride	NMR	NA	Dharmaratne *et al.* (2002)
Kava dry extracts	Kavain and total kavalactones/ routine quality control	None	NIRS	NA	Gaub *et al.* (2004)
Food supplements containing kava	Total kavalactones/regulatory control	Solution in ethanol, buffer addition	NMR	NA	Monakhova *et al.* (2013)

DMSO, dimethyl sulfoxide; GC, gas chromatography; HPLC, high-performance liquid chromatography; HPLC-DAD, high-performance liquid chromatography with diode-array detection; HPLC-FD, high performance liquid chromatography with fluorescence detection; HSCCC, high-speed counter-current chromatography; LC, liquid chromatography; MS, mass spectrometry; NA, not applicable; NIRS, near infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; v:v, volume:volume

considerably. Kava drinking initially produces a slight numbing of the tongue. Delayed effects have been described as relief of fatigue, reduction of anxiety, and production of a pleasant, cheerful, and sociable attitude in the drinker (WHO, 2007).

The consumption of kava is part of everyday life on islands such as Fiji, Tonga, and Vanuatu, and occurs during important events or social gatherings (Singh, 1992).

It is difficult to compare the psychopharmacological effects of kava between published studies as methods of preparation, means of ingestion, and the potency and quantity of dosages actually consumed vary considerably (Cairney *et al.*, 2002).

Kava bars, at which prepared kava can be purchased to drink on the spot or to take away, are an increasingly common feature throughout Oceania (McDonald & Jowitt, 2000).

(c) Non-traditional food use

Non-traditional kava products are marketed in Europe and North America typically as food or dietary supplements in tablet form (Morris & Avorn, 2003; Teschke & Lebot, 2011). Interestingly, these food supplements are often marketed over the internet (Morris & Avorn, 2003). In some countries this may be due to difficulties with regulatory acceptance. For example, in Europe this practice is illegal, but kava products are nevertheless available (Monakhova *et al.*, 2013).

(d) Cosmetic use

Kava extracts from various parts of the plant may be used as skin-conditioning agents in cosmetics. However, the USA Cosmetic Ingredient Review expert panel concluded that the available data were insufficient to support the safety of kava extracts for cosmetic use (Robinson *et al.*, 2009).

1.3.2 Dosage

(a) Medicinal use

The comminuted crude drug and extracts are used for oral use. Daily dosage for crude drug and extracts is equivalent to 60–210 mg of kavalactones (WHO, 2004). The recommended oral dose for use of commercial kava extracts as an anxiolytic is 50–70 mg of kavalactones, two to four times per day and, as a hypnotic, 150–210 mg in a single oral dose before bedtime (Bilia *et al.*, 2002b).

The pharmaceutical industry was primarily interested in the organic solvent (such as 95% ethanol or acetone) extracts of kava containing the organic compounds of commercial interest. Some marketed products, referred to as "synthetic," consist of a single kavalactone, L-kavain (WHO, 2007).

A review of standardized kava brands in the USA found an approximate equivalence of actual [measured] and labelled amounts of kavalactones in 13 products that listed amounts of constituents. Kavalactones per tablet or capsule ranged from 50 to 110 mg. Two brands that did not label amounts of constituents contained 10–15 mg per tablet or capsule (Ulbricht *et al.*, 2005).

Typical usage has ranged from 70 to 280 mg of kavalactones per day as a single bedtime dose or divided doses (60–120 mg of kavalactones per day). Many practitioners allegedly start at a lower dose and titrate up as needed (Ulbricht *et al.*, 2005).

(b) Traditional food and recreational use

Only rough estimations exist on the dosage of traditional food and in recreational use of kava. Heavy consumers may drink the equivalent of at least 610 g/week of kava powder, which, with an estimated kavalactone content of 12.5%, may equate to approximately 76 g of lactones per week or more than 50 times the recommended therapeutic dose (Cairney *et al.*, 2002).

In Arnhem Land, Australia, weekly per capita consumption was estimated as 145 g of powder for 1989–90 and 368 g of powder for 1990–91. When seven cups of 100 mL are consumed in 1 hour, about 3.8 g of lactones may be consumed. In a detailed review of the literature on weekly consumption levels and possible lactone contents, the estimations encompassed a wide variation from 39 to 1840 g of kava powder consumed, and from 4.1 g to 188.6 g of lactones consumed per week (Clough *et al.*, 2000).

Typical dosage of dried root or by decoction was reported to be 6–12 g per day (Morgan *et al.*, 2005).

(c) Non-traditional food use

The Dietary Supplements Label Database lists 11 products that contain kava as active ingredient in amounts of 60–1000 mg. Of the 11 products, 4 are listed as discontinued (NLM, 2012).

Kava food supplements, illegally sold over the internet in Germany, contained 8–10 mg of kavalactones per capsule (Monakhova *et al.*, 2013).

(d) Cosmetic use

The Cosmetic, Toiletry, and Fragrance Association (CTFA) provided a use concentration of 0.0001–0.01% for leaf/root/stem extract, and of 0.1% for root extract (Robinson *et al.*, 2009).

1.4 Production, sales, and consumption

1.4.1 Production

(a) Production process

Kava production including cultivation, diseases and pests, harvesting and processing has been reviewed by Singh (2004b).

(b) Production volume

Kava was one of the most extensively used herbal products in the USA in the 1990s (NTP, 2012). According to Morris & Avorn (2003), sales of kava were US$ 69 million in 2000. In 2003, 62 retail sites were identified that sold kava over the internet (Morris & Avorn, 2003).

In Australia, trade in kava rhizome in Arnhem Land was approximately 28 tonnes in 1992, and between 27 and 36 tonnes in 1997. At the end of 1999, by which time trade in kava was illegal, trade was estimated to be 20 tonnes, while in 2000 the trade was approximately 15 tonnes (Clough, 2003).

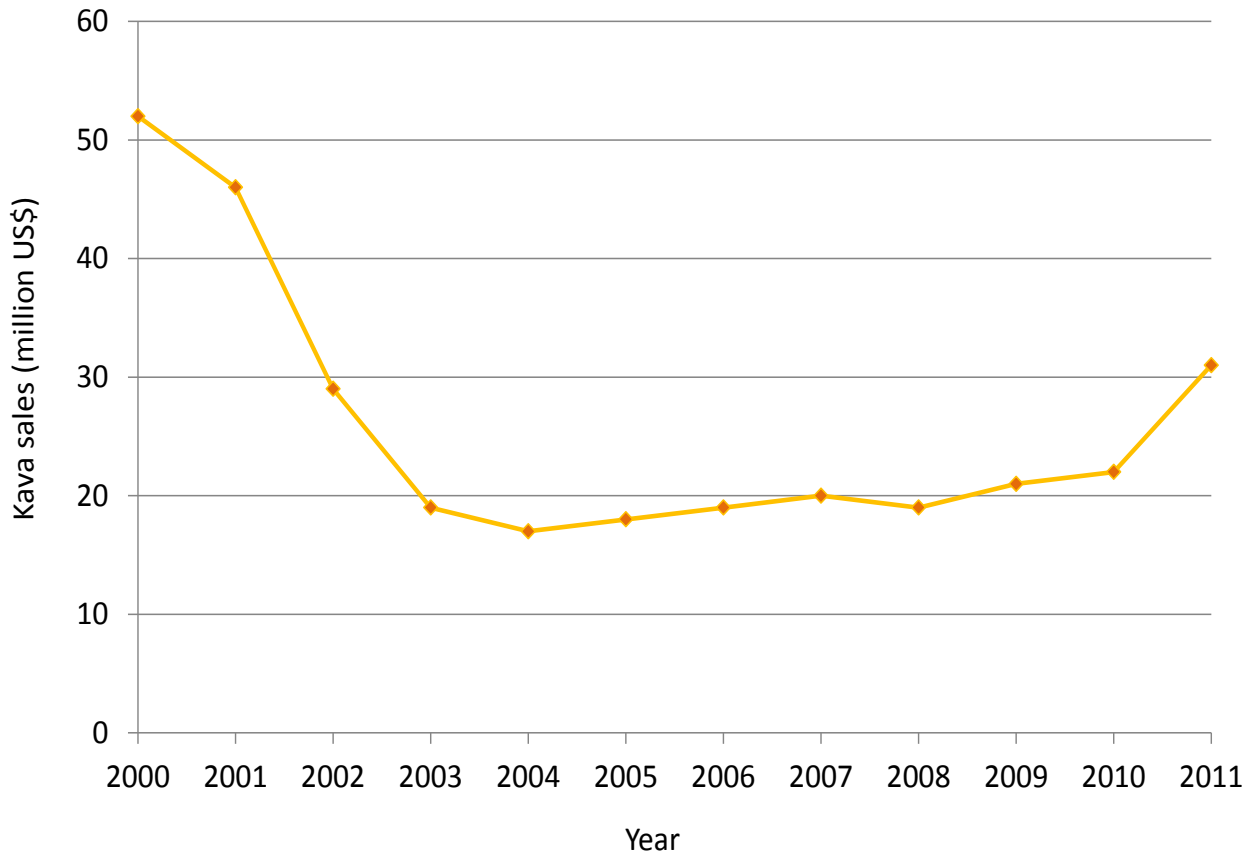
1.4.2 Sales

By the mid-1990s, North Americans, Europeans, and Australians had begun using kava products as an alternative medicine and herbal relaxant. Commercial kava bars promoted recreational kava drinking, which can often occur for extended periods. Drug stores and supermarkets offered a variety of kava products in pill, capsule, tea, and liquid form. In addition, powdered kava root was available by mail order from several internet sites. Most of this exported kava derived from Fiji and Vanuatu, and to a lesser extent, Samoa and Tonga (Lindstrom, 2004). Kava abuse has been reported, especially in Pacific Island nations, leading to significant health and social problems (McDonald & Jowitt, 2000; Rychetnik & Madronio, 2011).

Current use in North America, Europe, and Australia may have been influenced by regulatory measures (see Section 1.6) and reports of adverse events in the popular press.

According to the 2012 Nutrition Business Journal Annual Report, kava was the 36th best-selling dietary supplement in the USA in 2011. There has been a considerable decline in kava sales in the USA from US$ 52 million in 2000 to US$ 17 million in 2004. Sales remained at a similar level between US$ 18 and 22 million

Fig. 1.3 Sales of kava as a dietary supplement in the USA



Compiled by the Working Group from data from Nutrition Business Journal (2010, 2012).

during 2005–2011, and then increased to US$ 31 million in 2011 (Fig. 1.3; Nutrition Business Journal, 2010, 2012). Total global sales of kava (*Piper methysticum*) as an herbal supplement were US$ 8 million in 2012, and appreciable sales volumes occurred in the USA (US $3 million), Brazil (US$ 2 million), and Hungary (US$1 million) (IMS Health, 2012).

[The Working Group suggested that prohibition in some countries may have resulted in increases in unrecorded sales of kava, e.g. unrecorded individual imports, or illegal sales.]

1.4.3 Consumption

Consumers of products specified in Section 1.3 are exposed to kava. No literature about the degree of population-based exposure to kava was available to the Working Group. [The Working Group estimated that current exposure to kava was expected to be a fraction of what it was in the previous decade due to withdrawal of marketing authorization in many countries (see Section 1.6).]

Table 1.2 Guidelines for dried kava rhizome

Characteristic	Guideline
Content	Not less than 3.5% kavapyrones [kavalactones], as determined by IR spectroscopy
Identity tests	Macroscopic, microscopic and microchemical examinations, and TLC for the presence of characteristic unsaturated α-pyrones known as kavapyrones [kavalactones]
Microbiological purity, heavy metals, radioactive residues	Limits according to WHO guidelines on quality control methods for medicinal plants
Foreign organic matter	Not more than 2%
Total ash	Not more than 8%
Acid-insoluble ash	Not more than 1.5%
Water-soluble extractive	Not less than 5%
Loss on drying	Not more than 12%
Pesticide residues	Aldrin and dieldrin not more than 0.05 mg/kg. For other pesticides, general guidelines apply.

IR, infrared; TLC, thin-layer chromatography
From WHO (2004)

1.5 Occupational exposure

No specific studies on occupational exposure were available to the Working Group. It can be assumed that workers in kava production for food, cosmetic, or medicinal use may be exposed.

1.6 Regulations and guidelines

Several cases of liver damage have been associated with exposure to kava in Europe, and have led to withdrawal of the product license (NTP, 2012). Reviews on the cases of adverse effects potentially caused by exposure to kava have been compiled by Schmidt *et al.* (2005) (detailed analysis of 83 cases), as by WHO (2007) (analysis of 93 cases). Speculations about the causes of the adverse effects included the use of less expensive stem peelings in commercial materials instead of the usual peeled rhizomes (Teschke *et al.*, 2011).

Sales of kava have been suspended or withdrawn in several countries, including Australia, Canada, France, Germany, Spain, and Switzerland, and due to reported association with hepatotoxicity in humans (Russmann *et al.*, 2001, 2003; Campo *et al.*, 2002; De Smet, 2002; Parkman, 2002; Clough *et al.*, 2003; Humberston *et al.*, 2003; Teschke *et al.*, 2003; Ulbricht *et al.*, 2005; NTP, 2012).

Although sales of kava were not regulated or controlled in the USA in 2012 (NTP, 2012), the Food and Drug Administration (FDA) had issued a public warning in 2001 that kava might be be associated with serious liver damage, including hepatitis, cirrhosis, and liver failure (FDA, 2002). The regulatory action taken by various countries around the world from the year 2000 after concerns about hepatotoxicity is summarized in WHO (2007). Current regulatory status was summarized by Teschke & Lebot (2011), and included suggested chemical standards and agricultural standardizations. WHO (2004) provided some guidelines for the quality control of kava (see Table 1.2).

2. Cancer in Humans

Steiner (2000) investigated the association between cancer incidence and consumption of kava in an ecological study of six countries in the South Pacific. Exposure was estimated by a surrogate measure of consumption based on the number of kava plants under cultivation in

each country. Exposure estimates and data on cancer incidence for men in the 1980s were used in the analysis on the assumption that all kava produced before 1990 was consumed locally, and primarily by men. An inverse correlation was observed between the incidence of all cancers in men and estimated exposure, but no test of statistical significance or confidence intervals, was reported. [The Working Group considered this study as uninformative because of its ecological design, the use of crude measures of exposure and outcome, and inadequate assessment of the role of chance.]

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

In one study of oral administration, groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were given kava extract at a dose of 0 (corn oil vehicle, 10 mL/kg body weight, bw), 0.25, 0.5, or 1.0 g/kg bw per day by gavage, 5 days per week, for 105 weeks. The purity of the kava extract was 98.04% by high-performance liquid chromatography/ultraviolet (HPLC/UV) profiles. The extract contained 27% kavalactones identified as kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin. In males, the mean body weight of the dosed groups was similar to that in the control group. In females, the mean body weight of the group at 1.0 g/kg bw was 11% less than that in the control group after week 21. The mean survival time of male and female mice in the dosed groups was similar to that of the controls.

In males, the incidence of hepatoblastoma was significantly higher in the groups receiving the intermediate and highest dose, and had a significant positive trend. The incidence of hepatocellular carcinoma and hepatoblastoma (combined) was significantly higher in the group receiving the intermediate dose. The incidence of eosinophilic hepatocyte foci, a preneoplastic hepatocyte lesion, was significantly higher in the groups receiving the intermediate or highest dose.

In females, the incidence of hepatocellular carcinoma was significantly higher in the group receiving the lowest dose. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in the groups receiving the lowest and intermediate doses. The incidence of hepatocellular carcinoma and hepatoblastoma (combined) was significantly higher in the group receiving the lowest dose. [The Working Group noted that reduced body weight may have reduced one tumour response in females at the highest dose.] The incidence of eosinophilic hepatocyte foci was significantly higher in the group receiving the highest dose. The incidence of squamous cell hyperplasia of the forestomach was significantly higher in the groups receiving the lowest or intermediate doses (Behl *et al.*, 2011; NTP, 2012).

3.2 Rat

See Table 3.1

In one study of oral administration, groups of 49 or 50 male and 50 female F344/N rats (age, 6–7 weeks) were given kava extract at 0 (corn-oil vehicle, 5 mL/kg bw), 0.1, 0.3, or 1.0 g/kg bw per day by gavage, 5 days per week, for 104 (male rats) or 105 (female rats) weeks. The purity of the kava extract was 98.04% by HPLC/UV profiles. The extract contained 27% kavalactones identified as kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin. The mean body weight of the groups at 1.0 g/kg bw was 10% less than that of the control group after week 65 in males and after week 41 in females. The mean survival time for rats in the dosed groups was similar to that of controls for both sexes.

Table 3.1 Studies of carcinogenicity with kava extracts in mice and rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance[a]	Comments
Mouse, $B6C3F_1$ (M, F) 105 wk NTP (2012), Behl *et al.* (2011)	0 (control), 0.25, 0.5, or 1.0 g/kg bw by gavage in corn oil, 5 days/wk for 105 wk 50 M and 50 F/group (age, 5–6 wk)	Hepatocellular carcinoma: 3/50, 13/50*, 8/50, 8/50 (F) Hepatocellular adenoma or carcinoma (combined): 38/50, 39/50, 39/50, 40/50 (M); 10/50, 21/50*, 20/50**, 13/50 (F) Hepatoblastoma: 0/50*, 4/50, 9/50**, 12/50*** (M); 0/50, 0/50, 1/50, 0/50 (F) Hepatocellular carcinoma or hepatoblastoma (combined): 20/50, 21/50, 30/50*, 25/50 (M); 3/50, 13/50**, 9/50, 8/50 (F)	*$P = 0.007$ *$P = 0.015$ **$P = 0.036$ *$P < 0.001$ (trend) **$P = 0.002$ ***$P < 0.001$ *$P < 0.05$ **$P = 0.007$	Extract purity, 98.04% (HPLC/UV profiles), containing 27% kavalactones Mean body weight of females at 1.0 g/kg bw was 11% less than that in the vehicle-control group after wk 21
Rat, F344 (M, F) 104–105 wk NTP (2012), Behl *et al.* (2011)	0 (control), 0.1, 0.3, or 1.0 g/kg bw by gavage in corn oil, 5 days/wk for 104 (M) or 105 (F) wk 49 or 50 M, and 50 F (age, 6–7 wk)	Testis interstitial (Leydig) cell adenoma[b]: 37/49 (76%)*, 44/50 (88%), 49/50 (98%)**, 46/50 (92%)***(M)	*$P = 0.003$ (trend) **$P = 0.002$ ***$P < 0.001$	Extract purity, 98.04% (HPLC/UV profiles), containing 27% kavalactones Mean body weight of group at 1.0 g/kg bw was 10% less than that of the vehicle-control group after wk 65 (M) and wk 41 (F) No significant increase in the incidence of any neoplasm in females

[a] Poly-3 test

[b] Historical incidence in 2-year studies with administration by gavage with corn oil vehicle-control group (mean ± standard deviation): 176/199 (88.4% ± 8.6%), range 76–94%; all routes: 1053/1298 (81.1% ± 13.4%), range 54–98%

bw, body weight; F, female; HPLC/UV, high-performance liquid chromatography/ultraviolet; M, male; wk, week

In males, the incidence of testis interstitial (Leydig) cell adenoma was significantly higher in the groups at the intermediate or highest dose, and had a significant positive trend. [The incidence of this tumour in controls was low (76%) compared with that in historical controls (corn oil vehicle controls: range, 76–94%; all routes: range, 54–98%).] The incidence of renal pelvis transitional cell hyperplasia was significantly higher in the group receiving the highest dose. In females, the incidence of renal pelvis transitional cell hyperplasia was significantly higher in the groups at the highest or intermediate dose. There was no significant increase in the incidence of any neoplasm in females (Behl *et al.*, 2011; NTP, 2012).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

The metabolism of kava and individual kavalactones has been studied in humans (Duffield *et al.*, 1989; Köppel & Tenczer, 1991; Johnson *et al.*, 2003; Zou *et al.*, 2005). Demethylation and hydroxylation products were found in human urine after ingestion of kava extract (Duffield *et al.*, 1989), or its constituent kavain. The metabolites were mainly excreted as conjugates (Köppel & Tenczer, 1991).

Ten urinary metabolites were identified when kavain was given as a therapeutic oral dose of 200 mg to five healthy volunteers. The structures of kavain and its metabolites are shown in Fig. 4.1. The major metabolite was a hydroxydihydrokavain. Hydroxylation of the phenyl ring, reduction of the 7,8 double bond, hydroxylation of the lactone ring with subsequent dehydration, and opening of the lactone ring appeared to be the main metabolic pathways (Köppel & Tenczer, 1991).

Zou *et al.* (2005) identified a pyrone ring-opened product, 6-phenyl-3-hexen-2-one, a proposed metabolite of kava, as its mercapturic acid adduct, in urinary samples from two kava drinkers. This metabolite was possibly formed from enzymatic demethylation of 7,8-dihydromethysticin, followed by ring opening of the α-pyrone ring, and rearrangement (Zou *et al.*, 2005).

11,12-Dihydroxy-7,8-dihydrokavain-*o*-quinone and 11,12-dihydroxykavain-*o*-quinone, two electrophilic metabolites, were identified as glutathione conjugates when kava extract was incubated with human liver microsomes. The glucuronic acid and sulfate conjugates of these two urinary metabolites were detected in a human volunteer who ingested a single dose of a dietary supplement containing kava extract (about 90 mg of kavalactones) (Johnson *et al.*, 2003).

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

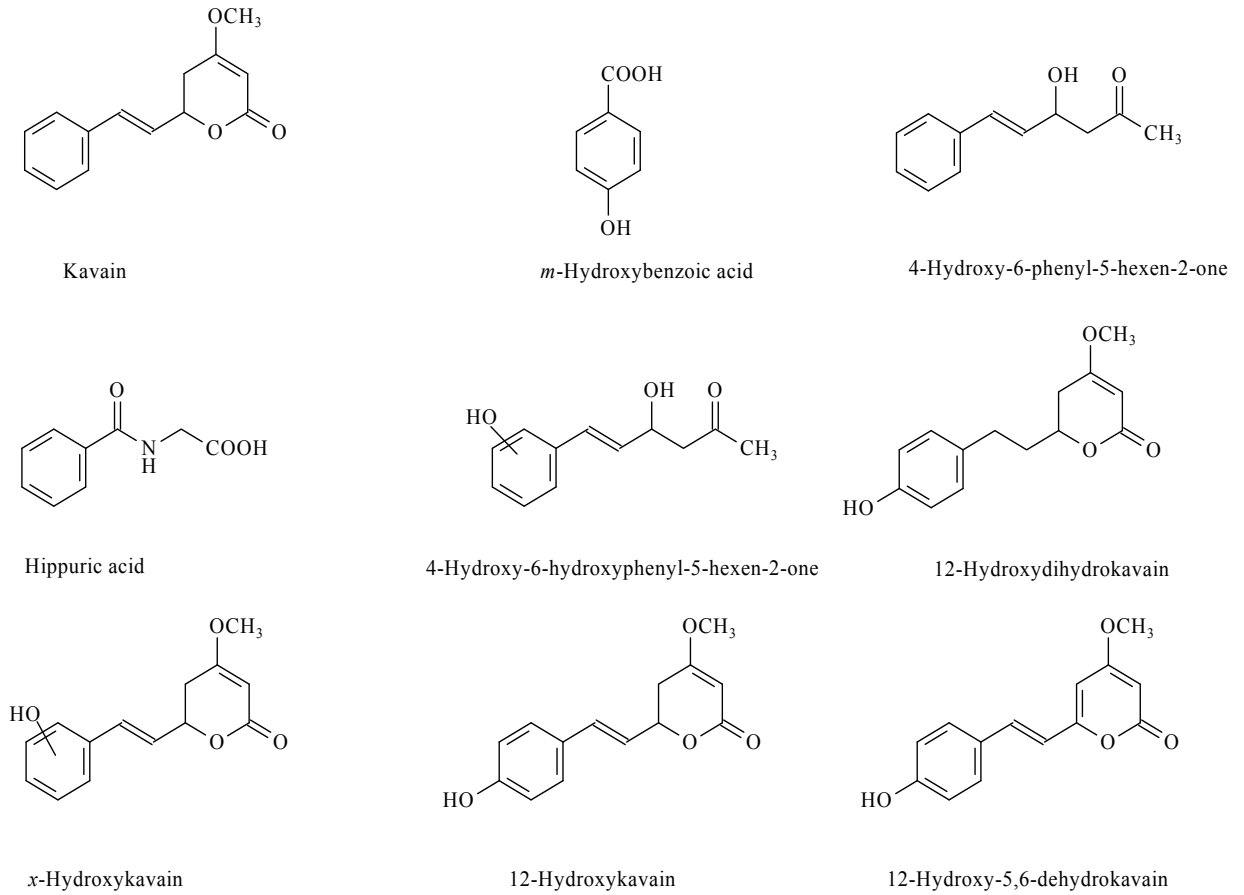
Few studies have been published on the absorption, distribution, and excretion of the constituents of kava (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, desmethoxyyangonin, and dihydroyangonin).

Kavain is rapidly absorbed from the gastrointestinal tract, distributed to tissues, and eliminated.

In male F344 rats given kavain at a single oral dose of 100 mg/kg bw, the maximum blood concentration of kavain was measured at 0.88 hours, after which plasma concentrations declined with a mean terminal half-life of 1.3 hours. The mean oral bioavailability of kavain in F344 rats was about 50% (Mathews *et al.*, 2005).

In male F344 rats given kavain orally for 7 days, kavain was primarily excreted in the urine, with about 77% recovered during the

Fig. 4.1 Structures of kavain and its eight identified metabolites



Compiled by the Working Group

72 hours after the last dose. Faecal excretion accounted for about 14% of the administered dose. Only 0.4% of the kavain was retained in the tissues, and kavain did not accumulate preferentially in any particular tissue. In addition, there were no differences in the pharmacokinetics of kavain when administered as a single dose or as repeated doses (Mathews *et al.*, 2005).

Oral absorption of kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin was investigated in mice (Meyer, 1967). Kavain and dihydrokavain were rapidly absorbed from the gastrointestinal tract (with a peak at 10 minutes), followed by methysticin and dihydromethysticin (30–45 minutes). Yangonin and desmethoxyyangonin were poorly absorbed, and rapid elimination occurred (Meyer, 1967; Robinson *et al.*, 2009).

In male F344 rats given an intravenous injection of kavain at a dose of 7 mg/kg bw, kavain was rapidly eliminated from the systemic circulation, with a terminal half-life of 0.63 hours. Systemic clearance and volume of distribution were 89 mL/minutes per kg and 2.70 L/kg, respectively, indicating that a significant amount of kavain was rapidly distributed out of the plasma into tissues and quickly cleared from the body (Mathews *et al.*, 2005).

Keledjian *et al.* (1988) observed a peak concentration at 5 minutes in brain for kavain and 7,8-dihydrokavain; the compounds were rapidly eliminated after intraperitoneal administration (100 mg/kg bw) of individual kava constituents in male Balb/c mice. The maximum concentrations of kavain and 7,8-dihydrokavain were 64.7 and 29.3 ng/mg wet brain tissue, respectively. The maximum concentrations of desmethoxyyangonin and yangonin were 10.4 and 1.2 ng/mg wet brain tissue, lower than those of kavain or 7,8-dihydrokavain. When kava extract was given intraperitoneally to male Balb/c mice, the maximum concentrations of kavain and yangonin increased in the brain, while the concentrations of 7,8-dihydrokavain and desmethoxyyangonin were similar to those measured after they were injected separately (Keledjian *et al.*, 1988).

(b) Metabolism

Rasmussen *et al.* (1979) investigated the metabolism of five kavalactones (kavain, dihydrokavain, methysticin, yangonin, and dihydroyangonin) in male albino rats. The individual kavalactones were administered orally (400 mg/kg bw) or intraperitoneally (100 mg/kg bw), the metabolites and the recovered parent substrate in the urine were then identified. Kavalactones were metabolized to several products via demethylation, mono- and dihydroxylation, and reduction and pyrone ring-opening (Rasmussen *et al.*, 1979; NTP, 2012).

With 7,8-dihydrokavain, large amounts of the parental compound were found in the urine. Nine metabolites were identified, with 12-hydroxydihydrokavain being the most abundant. About two thirds of the metabolites were hydroxylated forms, and one third of the metabolites were formed by scission of the 5,6-dihydro-α-pyrone ring (Fig. 4.2). The proposed metabolic pathways for 7,8-dihydrokavain are depicted in Fig. 4.2 (adapted from NTP (2012).

With kavain, a total of 10 metabolites were formed in very small amounts. Eight were determined structurally and two remained unidentified. Both hydroxylated and ring-opened products were formed (Fig. 4.1).

With methysticin, only small amounts of two metabolites (11,12-dihydroxykavain and 11,12-dihydroxydihydrokavain) were formed by demethylenation of the methylenedioxyphenyl moiety (Fig. 4.3).

Metabolites of yangonin and dihydroyangonin were formed via *O*-demethylation. No ring-opened products were detected (Fig. 4.4 and Fig. 4.5).

4.1.3 Effects on drug-metabolizing enzymes

Studies *in vivo* and *in vitro* have shown that kava extract and its constituents altered drug-metabolizing enzymes. Table 4.1 lists the major enzymes affected by kava.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

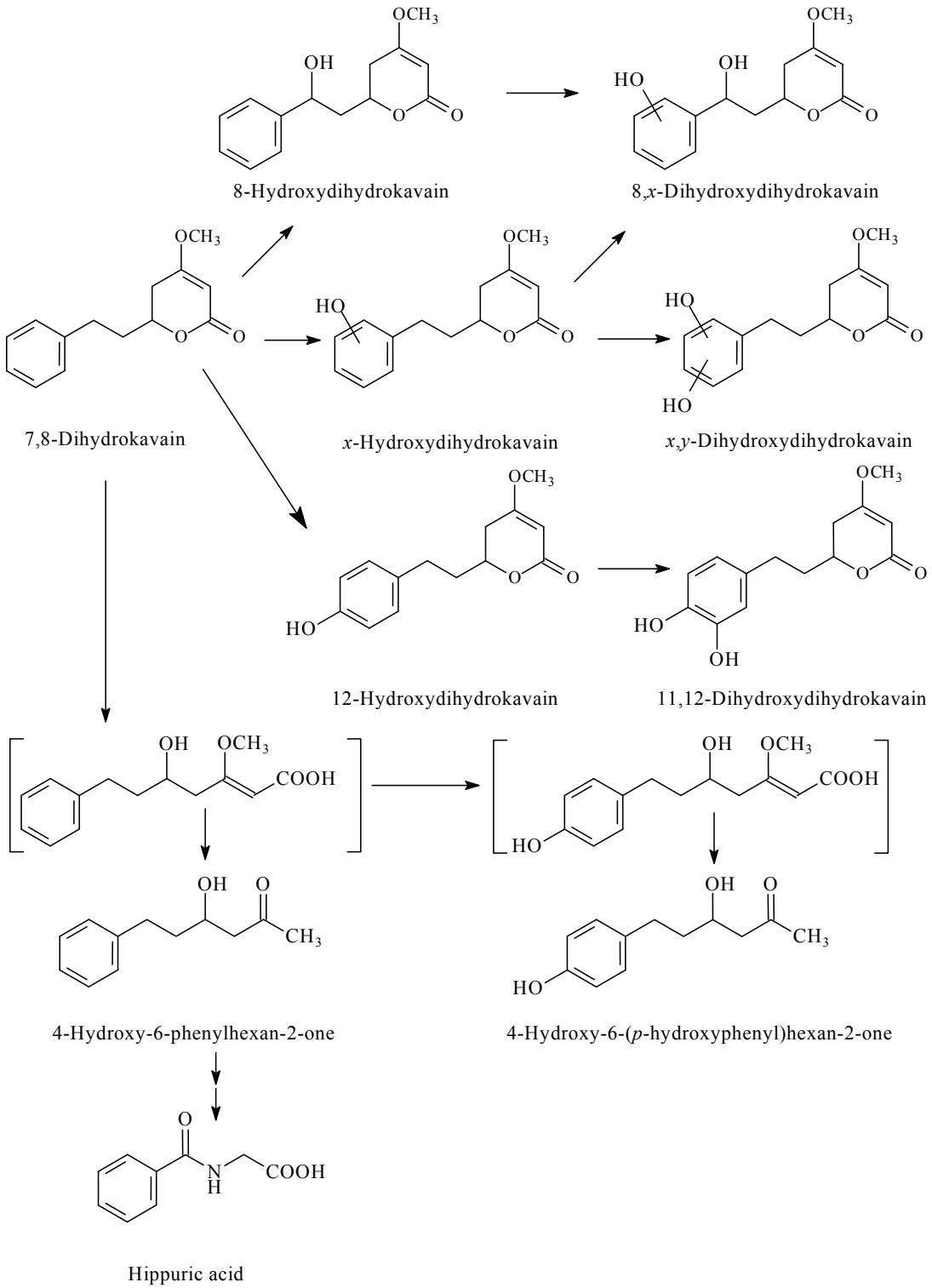
4.2.2 Experimental systems

See Table 4.2

(a) Mutagenicity

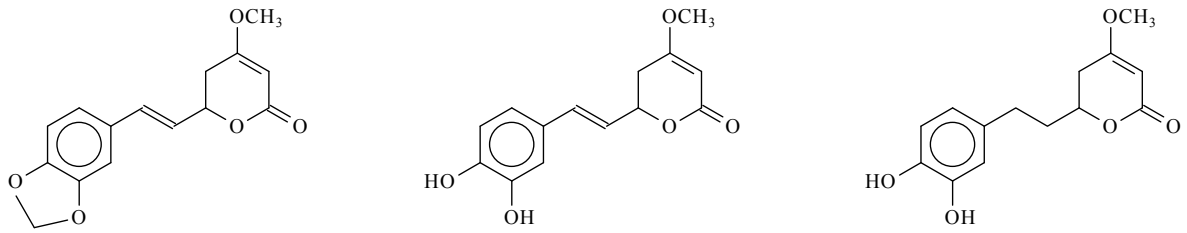
Kava extract (up to 10 000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA1535 or TA100, or *Escherichia coli* strain WP2 *uvr*A pKM101 with or without metabolic activation (rat liver S9) (NTP, 2012). In one trial out of three of which two of the results were negative, kava extract tested equivocal in TA97 with metabolic activation (NTP, 2012). Kava extracts were not mutagenic in an assay in L5178Y mouse lymphoma cells (Whittaker *et al.*, 2008).

Fig. 4.2 The proposed metabolic pathways for 7,8-dihydrokavain



Compiled by the Working Group using data from NTP (2012).

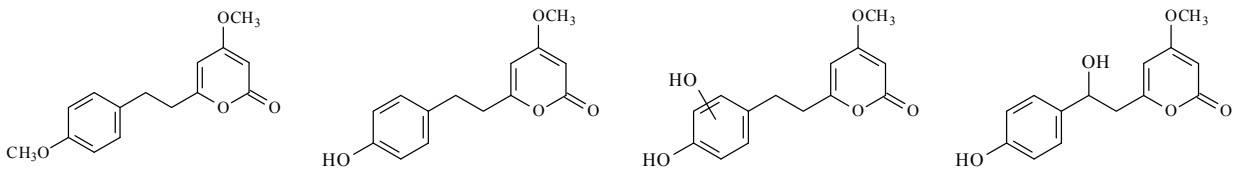
Fig. 4.3 Structures of methysticin and its two metabolites



Methysticin | 11,12-Dihydroxykavain | 11,12-Dihydroxydihydrokavain

Compiled by the Working Group using data from Fu *et al.* (2008)

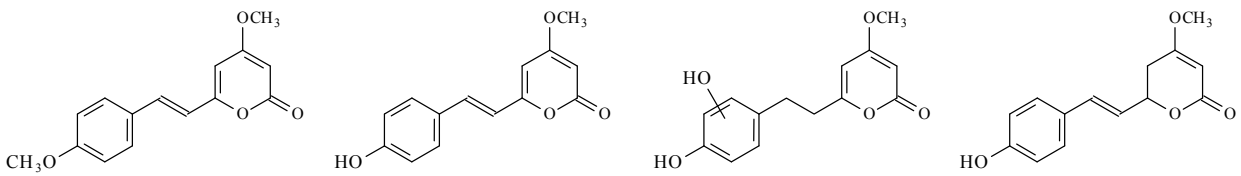
Fig. 4.4 Structures of 7,8-dihydroyangonin and its three metabolites



7,8-Dihydroyangonin | 12-Hydroxy-5,6-dehydro-7,8-dihydrokavain I | Dihydroxy-5,6-dehydro-7,8-dihydrokavain I II | Dihydroxy-5,6-dehydro-7,8-dihydrokavain II III

For the metabolites II and III the positioning of the second hydroxyl group (m, o or at C8) are uncertain.
Compiled by the Working Group using data from Fu *et al.* (2008)

Fig. 4.5 Structures of yangonin and its three metabolites



Yangonin | 12-Hydroxy-5,6-dehydrokavain | *x*,12-Dihydroxy-7,8-dihydro-5,6-dehydrokavain | 12-Hydroxy-kavain

Compiled by the Working Group using data from Fu *et al.* (2008)

Table 4.1 Effects of kava extract and kavalactones on metabolizing enzymes

Reference	Species or cell type	Kava preparation	Dose	Duration of treatment	Detection method	Result
In vivo						
Russmann *et al.* (2005)	Human	Aqueous extract	7–27 g of kavalactones per wk, oral	6 yr	Substrate turnover	CYP1A2 (inhibition)
Guo *et al.* (2010)	Mouse	Methanolic and aqueous extracts	0.125–2.0 g/kg bw per day, 5 days/wk, gavage	98 days	Gene expression	CYP4A10 (inhibition); CYP2A5, CYP2B20, CYP2C55, GSTA1, GSTA2 (induction)
Guo *et al.* (2009)	Rat	Methanolic and aqueous extracts	0.125–2.0 g/kg bw per day, 5 days/wk, gavage	98 days	Gene expression	CYP3A13, CYP17A1, ABCB9 (inhibition); CYP1A1, CYP1A2, CYP3A1, CYP3A3, ABCC3, NQO1, UGT1A6 (induction)
Yamazaki *et al.* (2008)	Rat	Kava extracts (not specified)	Kava extract (kavalactones, 380 mg/kg bw per day), gavage	8 days	Rat liver microsomes/ enzyme assay, gene expression, protein expression	CYP1A1, CYP1A2 (induction)
Lim *et al.* (2007)	Rat	Acetone kava leave extract	100 mg/kg/ bw per day, gavage	14 days	Protein expression	CYP1A2, CYP2E1 (induction)
Clayton *et al.* (2007)	Rat	Methanolic and aqueous extracts	0.125–2.0 g/kg bw per day, 5 days/wk, gavage	90 days	Protein expression	CYP2D1 (inhibition); CYP1A2, CYP2B1, CYP3A1 (induction)
Mathews *et al.* (2005)	Rat	Methanol or acetone kava extract	256 mg/kg bw, 1 g/kg bw, gavage	7 days	Rat liver microsomes/ enzyme assay	CYP2D1, 2C11 (inhibition); CYP1A2, 2B1, 2C6, 2D1, 3A1/2 (induction)
In vitro						
Li *et al.* (2011)	Hepa1c1c7	Methanolic and aqueous extracts, methysticin, 7,8-dihydromethysticin	Various concentrations to 25 μM of kava constituents or 6.25 μg/mL of kava extract	24 h	Cell-based enzymatic assay/enzyme assay, gene expression, protein expression	CYP1A1 (induction)
Mathews *et al.* (2005)	Human liver microsomes	Methanol or acetone kava extract, yangonin, dihydrokavain, methysticin, dihydromethysticin, composite kavalactones	1, 10, 100 μM	10 min	Recombinant protein/ enzyme assay	CYP2C9, 2C19, 2D6, 3A4 (inhibition); P-glycoprotein ATPase (inhibition)

Table 4.1 (continued)

Reference	Species or cell type	Kava preparation	Dose	Duration of treatment	Detection method	Result
Weiss *et al.* (2005)	P388 and P388/dx cell lines	Methanol aqueous kava extracts, kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin	Various concentrations (to the highest soluble concentration)	NR	Calcein uptake	ABCB1 (inhibition)
Zou *et al.* (2004)	Baculovirus/ insect cell system and cryopreserved human hepatocytes	Ethanol extract, methysticin, desmethoxyyangonin, yangonin	Various concentrations up to 100 µM	15–45 min	Recombinant enzyme/ enzyme assay	CYP1A2, 2C9, 2C19, 2E1, 3A4 (inhibition)
Côté *et al.* (2004)	Human liver microsomes/	Methanol, acetone, ethanol or aqueous kava extracts	Various concentrations up to 200 µg/mL	5 min	Enzyme assay	CYP3A4, CYP1A2, CYP2C9, CYP2C19 (inhibition)
Raucy (2003)	Primary human hepatocytes and HepG2	Kava extract (not specified)	100 µg/mL	48 h	Gene expression, report gene assay	CYP3A4 (induction)
Mathews *et al.* (2002)	Human liver microsomes/	Methanol or acetone extract, desmethoxyyangonin, methysticin, dihydromethysticin	Kava extract normalized to 100 µM kavalactones	15 min	Enzyme assay	CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP4A9/11 (induction)
Unger *et al.* (2002)	Baculovirus/ insect cell system	Methanol, acetone and ethyl acetate extracts	1–100 mg/mL	30 min	Recombinant enzyme/ enzyme assay	CYP3A4 (inhibition)
Zou *et al.* (2002)	cDNA human CYP isoforms	Methysticin, desmethoxyyangonin, dihydromethysticin, kavain, dihydrokavain	Various concentrations up to ~200 µM	30–45 min	Recombinant enzyme/ enzyme assay	CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (inhibition)

ABC, ATP-binding cassette; CYP, cytochrome; GST, glutathione-*S*-transferase; min, minute; NR, not reported; NQO, NAD(P)H quinone oxidoreductase; UGT, UDP glycosyltransferase; wk, week; yr, year

Table 4.2 Genetic and related effects of kava extract

Test system	Results[a]		Concentration or dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA98, TA1535, reverse mutation	–	–	10 000 µg/plate	NTP (2012)
Salmonella typhimurium TA97, reverse mutation	–	Equivocal in 1 out of 3 tests	10 000 µg/plate	NTP (2012)
L5178Y mouse lymphoma mutation assay	–	NR	300 µg/mL	Whittaker et al. (2008)
umu point mutation assay	+[a]	+[a]	2330 µg/mL extract 300 µM pure kavalactones	Jhoo et al. (2007)
Escherichia coli WP2 *uvrA*/pKM101, reverse mutation	–	–	10 000 µg/plate	NTP (2012)
Micronucleus induction in peripheral blood erythrocytes of male and female $B6C3F_1$ mice in vivo	–	NT	Up to 2.0 g/kg bw per day by gavage for 3 months	NTP (2012)

+, positive; (+), weakly positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; NR, not reported
[a] These data were not analysed statistically.

The only report of positive mutagenic activity with kava extracts (two positive results, but six negative results) involved the *umu* point mutation assay (Jhoo *et al.*, 2007). [The Working Group noted that these data were not analysed statistically.]

(b) Chromosomal damage

In male or female mice given kava extract at a dose of up to 2.0 g/kg bw per day by gavage for 3 months, there was no increase in the frequency of micronucleated normochromatic or polychromatic erythrocytes in blood (NTP, 2012).

4.3 Other mechanistic data relevant to carcinogenesis

Effects on hepatic cell physiology

Case reports of liver injury associated with kava intake have been described. The types of liver damage reported include fulminant hepatitis, necrosis, cirrhosis, and liver failure requiring liver transplantation or causing death (Russmann *et al.*, 2001; Bujanda *et al.*, 2002; Campo *et al.*, 2002; Brauer *et al.*, 2003; Gow *et al.*, 2003; Humberston *et al.*, 2003; Stickel *et al.*, 2003; Teschke *et al.*, 2003, 2008; Thomsen *et al.*, 2004).

4.4 Susceptibility

Genetic polymorphisms

Deficiency in CYP2D6, the major kavalactone-metabolizing enzyme, was detected in two patients with liver failure (Russmann *et al.*, 2001). Genetic polymorphism of CYP2D6 has a prevalence of 7–9% in Caucasian populations, but < 1% in Polynesian populations (Wanwimolruk *et al.*, 1998; Ingelman-Sundberg, 2005). Severe liver failure has not been observed in people using kava in the traditional way in islands in the South Pacific (Moulds & Malani, 2003; Anke & Ramzan, 2004).

4.5 Mechanistic considerations

Kava extract is not mutagenic based on the results of numerous studies of genotoxicity, including tests for mutagenicity in bacteria, induction of micronuclei *in vivo* (NTP, 2012), and the mouse lymphoma assay (Whittaker *et al.*, 2008). The reported carcinogenicity in mice is most probably mediated through nongenotoxic mechanisms.

5. Summary of Data Reported

5.1 Exposure data

The kava (or kava kava) plant *Piper methysticum* is a perennial tropical shrub that is widely cultivated in Oceania. The rhizome of the plant was originally used as an ingredient in local traditional drinks with psychopharmacological properties, and as traditional folk medicine. More recently, rhizome extracts have been used in medicinal products, food or dietary supplements, and cosmetics. Important chemical constituents of the resin contained in the kava rhizome are kavalactones, kavain being the major compound. The medicinal uses of kava supported by clinical data are short-term symptomatic treatment of mild states of anxiety or insomnia due to nervousness, stress, or tension. Use of kava was popular worldwide, but several case reports of liver damage associated with exposure to kava reduced sales, and caused kava to be banned in several countries.

5.2 Human carcinogenicity data

The Working Group was able to identify only one epidemiological study of cancer and kava consumption. This ecological study found an inverse correlation between all cancers in men and a proxy measure of kava consumption, but no confidence intervals or test of statistical significance were reported. The Working Group regarded the study as uninformative because the ecological design provided only weak support for causal inference at the individual level, the measures of exposure and outcome were crude, and the role of chance was not evaluated.

5.3 Animal carcinogenicity data

A kava extract was tested for carcinogenicity in one study in mice and one study in rats treated by gavage. In mice, the extract caused a significant increase in the incidence of hepatoblastoma in males, and of hepatocellular adenoma or carcinoma (combined), and hepatocellular carcinoma, in females. In male rats, the same extract caused a significant increase in the incidence of testis interstitial (Leydig) cell adenoma; however, the incidence in controls was low compared with that in historical controls. There was no significant increase in the incidence of any neoplasm in female rats.

5.4 Mechanistic and other relevant data

The major components of kava extract, kavalactones, are extensively metabolized in humans and experimental animals. Among the numerous metabolites are products from demethylation, hydroxylation, and ring-opening.

Kava extract gave negative results in several standard bacterial assays for mutation in the absence or presence of exogenous metabolic activation. Kavalactones gave negative results in most of these assays.

The reported carcinogenicity of kava in mice is most likely to be mediated through a nongenotoxic mechanism.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of kava extract.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of kava extract.

6.3 Overall evaluation

Kava extract is *possibly carcinogenic to humans (Group 2B).*

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