Review Article

Biological Activity, Hepatotoxicity, and Structure-Activity Relationship of Kavalactones and Flavokavins, the Two Main Bioactive Components in Kava (Piper methysticum)

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Kava (Piper methysticum Forst) is a popular and favorable edible medicinal herb which was traditionally used to prepare a nonfermented beverage with relaxant beneficial for both social and recreational purposes. Numerous studies conducted on kava have confirmed the presence of kavalactones and flavokawains, two major groups of bioactive ingredients, in this miraculous natural plant. Expectedly, both kavalactone and flavokawain components exhibited potent antianxiety and anticancer activities, and their structure-activity relationships were also revealed. However, dozens of clinical data revealed the hepatotoxicity effect which is indirectly or directly associated with kava consumption, and most of the evidence currently seems to point the compounds of flavokawains in kava were responsible. Therefore, our aim is to conduct a systematic review of kavalactones and flavokawains in kava including their biological activities, structure-activity relationships, and toxicities, and as a result of our systematic investigations, suggestions on kava and its compounds are supplied for future research.

1. Introduction

Piper methysticum Forst, popularly known as kava, is an edible and medicinal plant of shrub which has history of more than 2000 years. Given the purposes for religious occasions, medicinal purposes, and social gatherings [1–3], kava is particularly important for the indigenous people of the Pacific Rim and the Hawaiian Islands [4]. In the daily life of the South Pacific island people, the water infusion of kava root was used as a traditional beverage since ancient times for its sedative and calming effects, such as soothing the nerves, inducing relaxation and sleep, counteracting fatigue, and reducing weight [5, 6], and the dietary supplements of kava were easily obtained in some health food stores [7]. Similarly, in the Western world, pharmaceutical preparations of this herb were commonly applied for the treatment of anxiety disorders.

However, there was compelling evidence that kava consumption was related to some toxicities which led to its restriction or warning in many countries since 2002 [8, 9]. Several studies have reported a series of adverse health effects including kava dermopathy [10], hepatotoxicity [11, 12], and the disruption of cognition [13, 14] which were associated with kava consumption. Among those, kava hepatotoxicity was the most concerning adverse effect of kava consumption.

Although several of the published reviews have summarized the pharmacology, safety profiles associated with kava [3, 9, 15], however, over the past decades, dozens of studies which focused on the chemical constituents and biological activities of kava have been disclosed and some possible mechanisms of action have also been explored. Also, we found some scientific gaps still existed in the specific mechanism of its anticancer effect, as well as the
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2.1. Kavalactones. Kavalactones belong to lipophilic lactones with an α-pyrene skeleton typically 4-methoxy-2-pyrones, and aromatic styril or phenylethyl was substituted at the 6-position [16]. At present, 29 kavalactones, shown in Figure 1, have been isolated and identified. Kavalactones can be extracted from the rhizomes, roots, and root stems of the plant [9]. By employing gas chromatography-mass spectrometer (GC-MS) combined with high-performance liquid chromatography (HPLC) techniques, the extracting efficacies of different solvents (water, acetone, chloroform, methanol, ethanol, and hexane) on the contents of kavalactone constituents were determined [5], as Figure 2 shows. Seven major kavalactones, namely, methysticin (4), dihydromethysticin (5), kavain (6), 7, 8-dihydrokavain (7), desmethoxyyagonin (9), yangonin (10), and 5,6-dihydro-5,6-dehydrokavain (19), were obtained from the kava roots. It was found that acetone was the most effective solvent in terms of yield and quantities of kavalactone compounds obtained. The contents of seven major kavalactones including methysticin dihydromethysticin, kavain, 7, 8-dihydrokavain, desmethoxyyagonin, yangonin, and 5,6-dihydro-5,6-dehydrokavain were 1.2–14.4 mg/g, 3.2–51.9 mg/g, 3.3–41.5 mg/g 3.8–55.1 mg/g, 2.1–21 mg/g, 2.1–84.1 mg/g, and 1.9–27.1 mg/g, respectively [5].

A series of kavalactone dimers were also isolated and identified via extensive phytochemical investigation on the roots of kava [17–19]. By using classical chromatographic separation methods combined with spectrum identification techniques, a series of novel dimeric kavalactones, namely, diyangonins A (20), diyangonins B (21), diyangonins C (22), yangonindimers A (23), yangonindimers B (24), yangonindimers C (25), kavalactone A (26), aniba-dimer A (27), rel-, trans-3-bis[4-(4-methoxy-2-pyronyl)]cis-2, trans-4-diphenyl cyclobutane (28), and 6,6′-(3,4-diphenylcyclobutane-1,2-diyl) bis (4-methoxy-2H-pyran-2-one) (29), were isolated and elucidated from kava [17–19]. The chemical structures of compounds 20–29 are listed in Figure 1.

2.2. Flavokavins. The first three dihydrochalcones, namely, flavokavin A (30), flavokavin B (31), and flavokavin C (32), were isolated from the roots of kava by using the high-performance thin-layer chromatography (HPTLC) method [20]; followed by the pinosinotro chalcone (33), which was detected in kava roots for the first time by employing GC-MS and HPLC analysis [5]. Recently, two new flavanones, namely, pinosinotrobin (34) and 5,7-dimethoxyflavanone (35) [21], along with 5,7-dihydroxy-4′-methylx-6,8-dimethylflavonone (matteucinol,36) and 5-hydroxy-4′,7-dimethoxyflavanone (37) have been obtained via column chromatography (CC) and HPLC methods [5, 22]. The chemical structures of these flavanones are listed in Figure 3.

3. Biological Activities

Various uses and pharmacological properties of the isolated kavalactones and flavokavins from the rhizomes and roots of kava have been reported (Table 1; Figure 4). Lately, a published review has summarized the anti-inflammatory activity, neurological functions, and anticancer property of kava and its components [58]. To avoid repetition and exhibit our innovation, we supplied the details about the abovementioned activities of kavalactones and flavokavins, including the in vitro cell models and in vivo animal models, the methods of the experiments, the major findings, and the possible mechanisms, for example, the anti-inflammatory mechanisms of FKA, as Figure 5 shows, and the anticancer mechanisms of DHM, as Figure 6 described. All of them are exhibited in Table 1 and Figures 4–6.

4. Kava Hepatotoxicity

Kava became a well-known edible medicinal herb not only for its excellent activity but also for its controversy toxicity, and kava hepatotoxicity was the most concerning adverse effect of kava consumption [11]. Since the first case of kava hepatotoxicity was reported by in 1998 [59], more than 100 cases of severe liver injury following kava exposure have been identified all over the world. However, many of which were uncertain whether kava was responsible or it was caused by the other possible pathogenetic factors which were overlooked in reported cases of kava hepatotoxicity. For example, kava consumption involved concomitant ingestion of other agents with potential hepatotoxicity including other medications and/or alcohol [9]. Furthermore, the number of cases might be overstated as the types of liver injury noted include necrosis, drug-induced hepatitis, and cholestatic hepatitis [3]. It was interesting to note that, in the South Pacific, the adverse effect of liver damage was virtually absent during kava consumption. Cytochrome P450 2D6 (CYP2D6), an important enzyme which was necessary during drug metabolism, could also mediate the drug-drug interactions and, thus, might be responsible [60]. During the past years, suggestions and discussions have revealed the possible pathogenetic factors leading to the development of kava hepatotoxicity [11], and the details are given in the following.
| No. | Compounds                                      | R1 | R2 | R3 | R4 | C5-C6 | C7-C8 |
|-----|-----------------------------------------------|----|----|----|----|-------|-------|
| 1   | 11-Hydroxy-12-methoxydihydrokavain           | OCH₃ | OH |    |    |       |       |
| 2   | 7,8-Dihydro-5-hydroxykavain                  |    |    |    | β-OH|       |       |
| 3   | 11,12-Dimethoxydihydrokavain                 | OCH₃ | OCH₃|    |    |       |       |
| 4   | Methysticin (M)                               | OCH₂O |    |    |    |       |       |
| 5   | Dihydromethysticin (DHM)                     | OCH₂O |    |    |    |       |       |
| 6   | Kavain (K)                                    |    |    |    |    |       |       |
| 7   | 7,8-Dihydrokavain (DKH)                      | OCH₂O |    |    |    |       |       |
| 8   | 5,6-Dehydromethysticin                       |    |    |    |    |       |       |
| 9   | Desmethoxyyagonin (DMY)                      |    |    |    |    |       |       |
| 10  | Yangonin(Y)                                   | OCH₃ |    |    |    |       |       |
| 11  | 5,6,7,8-Tetrahydroxyagonin                   | OCH₃ |    |    |    |       |       |
| 12  | 5,6-Dihydroxyagonin                          | OCH₃ |    |    |    |       |       |
| 13  | 7,8-Dihydroxyagonin                          | OCH₃ |    |    |    |       |       |
| 14  | 10-Methoxyyagonin                            | OCH₃ | OCH₃|    |    |       |       |
| 15  | 11-Methoxyyagonin                            | OCH₃ | OCH₃|    |    |       |       |
| 16  | 11-Hydroxyyagonin                            | OCH₃ | OH |    |    |       |       |
| 17  | Hydroxykavain                                |    |    |    |    |       |       |
| 18  | 11-Methoxy-12-hydroxydehydrokavain           | OH  | OCH₃|    |    |       |       |
| 19  | 5,6-Dihydro-5,6-dehydrokavain (DDK)          | OCH₃ |    |    |    |       |       |

Figure 1: Chemical structures of compounds 1-29.
4.1. Different Sources and Parts of Kava for Practical Applications. Concerning the early history of kava, the lack of standard kava raw material might be the major factor, at least in some cases [61]. The different parts of the kava plant possessed different compounds, which showed different kava raw materials might contain different contents of the toxic constituents and then influenced the function of liver [11], for example, the substandard kava cultivars, different growth ages, using stem peelings replaced kava toots, rhizomes, or aerial parts of the kava plants (contains toxic alkaloids), and contamination of aflatoxicosis or other mould hepatotoxins [61]. Therefore, the botanical characteristics of the plant and the harvesting and storage conditions might be involved in the development of hepatotoxicity and triggering idiosyncratic reaction [11, 62].

4.2. Different Solvents Used for Kava Extraction. The next concern was whether the liver is damaged following kava consumption due to the solvent used for kava extract preparation or not [11]. Because the kavalactone and flavokavins contained in kava possessed different polarities, employing ethanol and acetone as solvents for the extraction of kava generally yielded high contents of kavalactone. As the higher portions of kavalactones was proved to be usually associated with liver failure, thus, using acetone as the extracted solvent might concentrate the toxic components. However, the results came from the World Health Organization (WHO) study which reported five live injury cases which were associated with the aqueous extracts of kava. Therefore, the solvent itself fails to involve in the overall pathogenesis of kava hepatotoxicity [11, 60].

4.3. Comedication, Overdose, and Prolonged Use. In many kava hepatotoxicity cases, other concurrent medications being taken by patients also existed; thus, it was uncertain whether the hepatotoxic reaction was initiated by kava itself or other drugs. Theoretically, the metabolic process of complicated drugs might be altered in some especially cases, and even the components themselves lacking evidences of hepatotoxicity might also exert hepatotoxic effects. Therefore, at least in some clinical cases, the interaction between kava and drug might be a potential factor for the hepatotoxicity [11]. Furthermore, prolonged kava treatment as well as overdose of kavalactones should not be overlooked [63, 64]. It was disclosed that nonadherence to medication was a common matter but not unique for kava treatment. However, at present, there are no studies that focus on the abovementioned subjects. Therefore, it was not available to answer the issue of kava hepatotoxicity that might be related with prolonged and overdose, and further experimental assessment was necessary.

4.4. Toxic Constituents, Metabolites, and Contaminations. Other unknown toxic components, the contaminations derived from various kava extracts, and storage process could not be excluded for the moment, for example, the piperidine alkaloid pipermethystine in aerial parts of the kava plants, the contamination of aflatoxicosis or other mould hepatotoxins [11, 65] during long time, and improper storage. It was proved that the alkaloid pipermethystine could induce liver cell death by glutathione (GSH) depletion and modulate MAPK and NF-κB signaling pathways in vitro [66]. However, other in vivo experimental animal studies obtained the converse results, which failed to cause any liver damage during alkaloid pipermethystine treatment. Therefore, it was uncertain that pipermethystine had the responsibility between kava and hepatotoxicity [61, 66, 67]. In addition, FKB has been considered as a possible pathogenic factor for human kava hepatotoxicity [61, 68]. It could induce cell apoptosis in hepatoblastoma (HepG2) (LD$_{50}$ = 15.3 ± 0.2 μM) and L-02 (LD$_{50}$ = 32 μM) cells via inducing oxidative stress, reducing the depletion of glutathione and inhibiting the I-κB kinase (IKK) activity in vitro [69]. FKB, meanwhile, induced hepatic damage by inhibiting NF-κB transcriptional activity in vivo [61, 68, 69]. Furthermore, kava hepatotoxicity also involved concomitant ingestion of other agents such as alcohol; thus, the metabolic interactions of kava with alcohol might also be a possible mechanism [70].

5. The Investigation of the Structure-Activity Relationship (SAR)

The structure-activity relationship study is a widely used and well-established method for the early drug discovery stage. The structural-based activity information was usually employed to screen for or optimize compounds to achieve drug-like properties [71]. Kavalactones and flavokavins possessed the unique pharmacological effects including the efficacy and side effects, which were all directly related to their structures [72]. Recently, different synthetic approaches of kavalactones, as well as the key
Table 1: The pharmacological activities of kavalactones and flavokavins in kava.

| S. no. | Activity/disease                                              | Active molecule(s) | Model system | Methods/dosage                                                                 | Result or major finding                                                                 |
|-------|---------------------------------------------------------------|-------------------|--------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| 1     | Sedative property                                             | Kavain            | Male Wistar rats | In vivo, Kavain 10, 30, and 100mg/kg, p.o. suspended in 0.5% carboxymethyl cellulose solution | (i) Shortened the sleep latency with kavain at doses of 30 and 100mg/kg  
(ii) Decreased the awake time with kavain at a dose of 3mg/kg  
(iii) Increased the nonrapid eye movement (non-REM) sleep time with kavain at doses of 30 and 100mg/kg  
(iv) No significant effects in total REM sleep time with kavain at any used doses  
(v) Increased the delta activity during non-REM sleep using kavain at doses of 30 and 100mg/kg |
| 2     | Anti-inflammatory activity                                     | Kavain analogue (Kav001) | Mouse bone marrow macrophages (BMM) and THP-1 cells; wild-type (WT) mice | In vitro: Enzyme-linked immunosorbent assay (ELISA), western blot analysis. In vivo: 4mg/kg. Enzyme-linked immunosorbent assay (ELISA).  
(i) Kav001 significantly inhibited P. gingivalis-induced CAIA/endotoxic shock  
(ii) Kav001-treated mice or macrophages quickly initiated their immune system to protect the host (mouse or cells) from P. gingivalis and LPS-induced TNF-α secretion via induction of B-cell lymphoma 6 (Bcl-6) and reduction of LITAF expression |
| 3     | Anti-inflammatory activity                                     | Flavokawain A (FKA) | RAW 264.7 cells | Western blot analysis, enzyme-linked immunosorbent assay (ELISA), electrophoretic mobility shift assay (EMSA), and transient transfection and luciferase assay  
(i) Flavokawain A inhibited inducible NO synthase (iNOS) and cyclooxygenase (COX-2) expression and subsequent production of NO and prostaglandin E2 (PGE2)  
(ii) Flavokawain A inhibited LPS-induced NF-κB and amphipathic protein 1 (AP-1) activation  
(iii) Flavokawain A inhibited the production of proinflammatory cytokines, such as TNF-α, interleukin-1β (IL-1β), and IL-6 |
| 4     | Periodontitis                                                  | Kava-241 compound | RAW 264.7 cells | Kava-241 40mg/kg. In vitro, enzyme-linked immunosorbent assay (ELISA) and cytotoxicity assay  
(i) Kava-241 treatment was associated to reduced cell death than kava treatment (p < 0.05)  
(ii) Both kava-241 treatment and prevention reduced alveolar bone loss (by 36.98% and 39.05%, respectively) |
| 5     | Rheumatoid arthritis (RA)                                     | Kava-241 compound | Pathogen-free DBA1/BO male mice | In vivo: Western blot analysis, enzyme-linked immunosorbent assay (ELISA), clinical inflammation score, and radiological analysis  
(i) Kava-241 reduced inflammatory cell recruitment and osteoclast activation  
(ii) Kava-241 treatment of P. gingivalis-infected BMMs reduced TNF-α secretion in a dose-dependent manner (40% decrease for 20μg/ml, 70% for 100μg/ml, and 90% for 200μg/ml) |

Figure 3: Chemical structures of compounds 30–37.
| Table 1: Continued. |
|-------------------|-------------------------------------------------|
| Activity/Compound | Action/Involvement | Mechanism/Method | Result/Outcome |
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| | | | |
| Flosfructi (FF) | | | |
| | | | |
| Human colon adenocarcinoma HT-29 and human mammary MCF-7 cells | | | |
| | | | |
| Cell viability assay, transwell invasion assay, and wound healing assay | | | |
| | | | |
| In vitro | | | |
| | | | |
| DHM | | | |
| | | | |
| Induced dorsal cutaneous melanoma growth in C3H mice | | | |
| | | | |
| In vivo | | | |
| | | | |
| DHM | | | |
| | | | |
| Increased intraocular melanoma growth in C3H mice | | | |
| | | | |
| In vivo | | | |
| | | | |
| Flosfructi (FF) | | | |
| | | | |
| Reduced dorsal cutaneous melanoma growth in C3H mice | | | |
| | | | |
| In vivo | | | |
| | | | |
| Flavokawain B (FKB) | | | |
| | | | |
| Reduced hepatocellular carcinoma growth in C3H mice | | | |
| | | | |
| In vivo | | | |
| | | | |
| Flavokawain C (FKC) | | | |
| | | | |
| Reduced carcinogen-induced hepatic tumor multiplicity in male and female B6C3F1 mice | | | |
| | | | |
| In vivo | | | |
| | | | |
| Flavokawain A (FKA) | | | |
| | | | |
| Reduced hepatocellular carcinoma growth in C3H mice | | | |
| | | | |
| In vivo | | | |
| | | | |
| Flavokawain B (FKB) | | | |
| | | | |
| Reduced hepatocellular carcinoma growth in C3H mice | | | |
| | | | |
| In vivo | | | |
| | | | |
biosynthetic enzymes of the kavalactone and flavokavain were reported [73, 74]. However, the difficulty of biosynthetic and chemical synthesis hindered the therapeutic use of kavalactones and flavokawains in both laboratory experiments and clinical trials [74]. In order to improve the efficacy and pharmaceutical properties of kavalactones and flavokavins, further medicinal chemistry optimization is needed.

5.1. Kavalactone Analogues. Lately, it was explored that kavalactone analogues exhibited in vitro anthelmintic activities against *Haemonchus contortus* larvae [75]. Through the chemical modifications of 2-, 3-, and 4-substituent on the pendant aryl ring (Figure 7), two kavalactones (yangonin and desmethoxyyangonin) and 17 analogues were synthesized. Among these analogues, compounds with 4-trifluoromethoxy, 4-phenoxy, 4-difluoromethoxy, and 4-N-morpholine substitutions showed convinced anthelmintic activities (IC50 < 8.9 μM) which were superior to desmethoxyyangonin (IC50 = 37.1 μM) and yangonin (IC50 = 15.0 μM) and, thus, provided an opportunity for developing novel anthelmintic agents [75].

Besides kavalactone, kavain analogues were also designed and synthesized through chemical modifications. The results of pharmacodynamic tests showed that the synthesized compounds possessed anti-inflammatory [25, 27, 28, 72, 76] and analgesic activities [77]. Kava-241, a kavain-derived compound, showed convinced efficacy in the prevention or treatment of advanced periodontal inflammation and related alveolar bone destruction in vitro and in vivo [27, 28] and, thus, might be a promising therapeutic agent against periodontal diseases in the future. Kav001, another kavain analogue, was designed and synthesized through optimizing the biological activity and structural physicochemical properties of kavain [24, 25]. Expectedly, kav001 displayed stronger analgesic activity than kavain [77].

5.2. Flavokawain Derivatives. Chalcones, an α,β-ununsaturated ketone, was explored generally due to its simple chemistry structure, ease of synthesis, diversity of substituents, and wide range of biological activities [78, 79]. Flavokawain was a kind of chalcones which was widely occurring in plants [78]. Through chemical modifications of the A-ring (R1 site)
and B-ring (R2, R3, and R4 site) (Figure 8), several flavokawain derivatives were designed, synthesized, and characterized. The anticancer properties of flavokawain in kava have been estimated due to the presence of the α, β-unsaturated ketone part through the structure-activity relationship studies of flavokawain derivatives [80]. The presence of electron-withdrawing and electron-donating groups could influence the effects of the α, β-unsaturated system and then cause the change of cytotoxicity [81]. Meanwhile, the presence of a hydroxyl group on the A-ring, rather than the B-ring, made the flavokawain derivatives more stable [80, 81]. Furthermore, effects of different functional groups were studied via substituent modification of the ortho, meta, and para positions on the B-ring. It was well established that the steric hindrance played a key role in the activity of flavokawain derivatives, which might exert cytotoxicity against cancer cell lines [82, 83]. The structure-activity relationship studies of flavokawain derivatives indicated that trimethoxy of the A-ring showed the most convinced cytotoxicity and selectivity, followed by the modification of the meta position on the B-ring and the substitution of halogen groups [82]. For example, (E)-1-(2′-hydroxy-4′,6′-dimethoxyphenyl)-3-(4-methylthio) phenyl prop-2-ene-1-one (FLS), a flavokawain derivative, showed good selectivity against the breast cancer MCF-7 cell line [84].

6. Kava Metabolism

The pharmacokinetics and pharmacodynamics studies of kava in humans were carried out by means of experiments involving self-medication [85]. In humans, kavalactones as well as their metabolites were generally eliminated in the urine and feces, and the peak plasma levels usually occur around 2 h after ingestion, with a half-life of about 9 h. Orally administered kava water extracts were excreted mostly unchanged into urine [86]. The metabolism of kavain studied by the human liver cell-line Hep-G2 [87] or human serum and urine [85] disclosed the metabolites of kava including p-hydroxykavain, p-hydroxy-7,8-dihydrokavain,
5,6-dehydrokavain, 6-phenyl-5-hexen-2,4-dione [85], p-hydroxy-5,6-dehydrokavain, and 6-phenyl-3-hexen-2-one [88]. In rats, approximately 50% to 75% of kavalactones were excreted as glucuronide and sulphate conjugates in the urine and 15% was in the bile [89–91]. The most frequent metabolic pathways for kavalactones in humans and rats included hydroxylation of the C-12 in the aromatic ring, hydroxylation and cleavage of the lactone ring with subsequent dehydration, reduction of the 7,8-double bond, demethylation of the 4-methoxyl group, reduction of the double bond at carbons in positions 3 and 4 (to form a saturated pyrone ring system), and demethylation of the 4-methoxy group in the α-pyrene ring or of the 12-methoxy substituent in yangonin [89, 90, 92].

**Figure 6:** Proposed diagrams of DHM-induced G0/G1 phase arrest and apoptosis through phosphoinositide 3-kinase (PI3K)/nucleotide-oligomerization domain-like receptor subfamily C3 (NLRC3) signaling pathway inhibition in colorectal cancer cells.

**Figure 7:** The structure modification of kavalactone.

5,6-dehydrokavain, 6-phenyl-5-hexen-2,4-dione [85], p-hydroxy-5,6-dehydrokavain, and 6-phenyl-3-hexen-2-one [88]. In rats, approximately 50% to 75% of kavalactones were excreted as glucuronide and sulphate conjugates in the urine and 15% was in the bile [89–91]. The most frequent metabolic pathways for kavalactones in humans and rats included hydroxylation of the C-12 in the aromatic ring, hydroxylation and cleavage of the lactone ring with subsequent dehydration, reduction of the 7,8-double bond, demethylation of the 4-methoxyl group, reduction of the double bond at carbons in positions 3 and 4 (to form a saturated pyrone ring system), and demethylation of the 4-methoxy group in the α-pyrene ring or of the 12-methoxy substituent in yangonin [89, 90, 92].

**Figure 8:** The structure-activity relationships of flavokawain.

### 7. Conclusions and Future Perspectives

Kava is a magical plant composed of various constituents, and furthermore, it possessed anxiolytic relaxant effects in the treatment of anxiety disorders and also exhibited the potential activities in cancer prevention and therapy. Phytochemical investigations on kava plant have resulted in the isolation and identification of at least 56 compounds. Among them, kavalactones and
dihydrochalcones were found to be the most widely studied chemical classes. In the last two decades, the separation and determination methods of kava extractions have gone through several technological innovations. So far, many new techniques were also developed for the qualitative and quantitative analysis of kava. However, more efficient and effective analytical methods are needed to determine the content of bioactive constituents and other unknown compounds on kava quality assessment due to the safety concerns of hepatotoxicity and other adverse effects [93, 94]. In summary, the possible pathogenic factors leading to the occurrence of kava hepatotoxicity were as follows: (1) the quality of kava raw material might be the major factor [61]; (2) concomitant ingestion of other drugs with potential hepatotoxicity [9]; and (3) the other unknown toxic components deriving from different kava extracts [11]. Research of kava hepatotoxicity faced multiple challenges because of the numerous compounds contained in kava extracts and limited number of affected patients [61]. Therefore, more clinical and experimental studies are needed to increase the knowledge of this field, and then, the relationship between kava and hepatotoxicity can be elucidated in the future [70].

A number of studies have reported the anticancer activity of kava extraction or the isolated individual components. The most investigated compound of kava was found to be flavokavain B followed by flavokavain A, which all belong to the chalcone family but possess different substituents on their aryl rings. The biological activities of chalcones were associated with the presence of a double bond in conjugation with carbonyl functionality [95, 96]. The mechanism of antiproliferative effect of kava was believed to be related with cell cycle arrest, induced apoptosis [97], and autophagy [42]. However, the role of autophagy was complex during the cancer therapy. As induced autophagy through the bioactive constituents of kava might become an attractive approach for cancer prevention and therapy in the future [44, 48], more investigations are required to identify the mechanism involved in this process. Meanwhile, the anticancer activity of kava was mainly focused on in vitro assessment, and only parts of studies were performed using in vivo models; current evidence from numerous clinical trials suggested the plant of kava was not sufficient to perform effective treatment for GAD. Therefore, future studies should be designed to fulfill these gaps. In order to give further information on the development of a new anticancer drug, more research is needed in the area of kava toxicity to explore the mechanisms of action on treat cancers, in the investigation of kava structure-activity relationship, and in the metabolism of kava. In summary, more clinical trials are needed to assess the effect of kava for treating GAD and the efficacy of kavalactones and flavokavains in treating cancers, and rational establishment of kava quality specifications will be beneficial for the general usages of kava. These reviews highlight areas for further research of kava constituents in the prevention and treatment of clinical diseases.

**Abbreviations**

ACC: Adenoid cystic carcinoma
ALL: Acute lymphoblastic leukemia
BMM: Bone marrow macrophages
COX: Cyclooxygenase
CRC: Colorectal cancer
DHM: Dihydromethysticin
ELISA: Enzyme-linked immunosorbent assay
FKA: Flavokavain A
FKB: Flavokavain B
FKC: Flavokavain C
GAD: Generalized anxiety disorder
HPLC: High-performance liquid chromatography
LPS: Lipopolysaccharide
MCF: Michigan cancer foundation
MTT: Methyl thiazolyl tetrazolium
NF-κB: Nuclear factor-κB
PCR: Polymerase chain reaction
PI3K: Phosphoinositide 3-kinase
Skp2: S phase kinase-associated protein 2
Tca: Thyroid cancer
TGF: Transforming growth factor
TNF-α: Tumor necrosis factor-α
TRAMP: Transgenic adenocarcinoma of the mouse prostate
WT: Wild type.

**Data Availability**

No data were used to support this study.

**Conflicts of Interest**

The authors have no conflicts of interest.

**Authors’ Contributions**

Yingli Wang, Chao Su, and Bo Zhang contributed equally to this work.

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