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REVIEW ARTICLE

Title: The protective effects of Kava (Piper Methysticum) constituents in cancers: a systematic review

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Key words: kava, piper methysticum, cancer, flavokavains, oral cancer

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Abstract

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Background: Kava is a beverage made from the ground roots of the plant Piper Methysticum and has long-held a significant place within Pacific island communities. Active compounds extracted from kava, and secondary metabolites, include kavalactones, chalcones, cinnamic acid derivatives and flavanones. It is thought that components of kava may exert an anti-proliferative effect through cell cycle arrest and promotion of apoptosis.

Methods: We conducted a systematic review to summarize available evidence of the anti-cancer effects of kava components, and investigate their potential use for oral squamous cell carcinoma (OSCC) treatment. Eligible studies were identified through a comprehensive search of OVID EMBASE, OVID MEDLINE and Web of Science, as at April 2018.

Results: Of 39 papers that met the inclusion criteria, 32 included in-vitro models and 13 included animal studies. A total of 26 different cancers were assessed with 32 studies solely assessing epithelial cancers, 6 mesenchymal cancers, and 1 study including both. There was only one report assessing an OSCC cell line. Anti-proliferative properties were demonstrated in 32 out of 39 papers. The most researched constituent of kava was flavokavain B followed by flavokavain A. Both were associated with increased expression of pro-apoptotic proteins and decreased expression of anti-apoptotic proteins. Further, they were associated with a dose-dependent reduction of angiogenesis.

Conclusion: There was heterogeneity of study models and methods of investigation across the studies identified. Components of kava appear to present an area of interest with chemo-therapeutic potential in cancer prevention and treatment, particularly for epithelial neoplasms. To date, there is a paucity of literature of the utility of kava components in the prevention and treatment of oral squamous cell carcinoma.

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Introduction

Kava is a beverage made from the ground roots of the plant *Piper Methysticum* (Fig. 1), directly translated as "intoxicating pepper". The word 'Kava' originates from Tongan and Marquesan and means "bitter".[1] Common names of Kava include: Kava; Kava-kava; Ava-ava; Antares; Ava; Ava pepper; Ava root; Awa; Fijian kava; Gea; Gi; Grog; Kao; Kava kava rhizome; Kava root; Kavapiper; Kavapyrones; Kavarod; Kavekave; Kawa; Kawa kawa; Kawa pepper; Kawa Pfeffer; Kew; Macropiper latifolium; Malohu; Maluk; Maori kava; Meruk; Milik; Pepe kava; Rhizoma piperis methystici; Sakaua; Sakau; Tonga; Yagona; Yangona; and Yongona.

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Kava has long-held a significant place within Pacific island communities, being consumed for more than 2,000 years by individuals of Polynesian, Micronesian and Melanesian descent.[2]

The kava beverage is commonly drunk for social, ceremonial and medicinal purposes, acting as a muscle relaxant and inducing sleepiness and relaxation.[3] Kava plays a valuable role as an item for exchange within political, religious and economic spheres. Traditional regulations existed on kava usage regarding gender, social class, religion and age.[4] The use and cultivation of Kava has extended from traditional to recreational use, particularly in the last couple of decades.[4,5] South Pacific islanders can consume Kava on a daily basis. Pacific island economies are reliant upon Kava both as a means of traditional subsistence and as a contemporary cash-crop and valuable export commodity.[4]

Recreational Kava usage has reached regions of Australia and New Zealand partly due to migration of Pacific Islander communities to these areas.[6] Kava was also introduced as an alcohol alternative to Australian Aboriginal communities residing in Arnhem Island in 1982 in an attempt to reduce alcohol-related harm in the community.[7,8] The import and purchase of Kava in Australia was unregulated until 6th of April 1998. The National Code of Kava Management's standard 2.6.3, [9] enabled individual states and territories to implement more restrictive measures. Revelation of hepatotoxicity risk followed safety assessments of adverse changes associated with excessive consumption.[9] WHO's safety review of Kava has held concerns over the introduction of non-traditional preparations of kava into "exposure regions" such as Arnhem Island, in the Northern Territory where excessive consumption occurs. Currently, import, advertising and sale of Kava in Australia and New Zealand is strictly controlled under the Customs (Prohibited Imports) Regulations Act.[10] Commercial importations are no longer allowed, with the exception of medical or scientific purposes and trafficable quantities for those without a license or permit is 2 kg.[10]

Kava is traditionally prepared using the rhizome of the plant, which can be used fresh or dried. If using fresh preparations, the roots are macerated and the resultant juices mixed with other solvents such as coconut milk or water. The more common style of consumption is obtained by adding water to finely ground kava roots followed by filtration to obtain the desired beverage.[11] Fresh preparation produces a stronger beverage than when prepared from dried starting material.[12]

Active compounds extracted from kava include 18 kavalactones, three chalcones, cinnamic acid derivatives and flavanones.[13] Recent studies have identified 30 secondary metabolites, expanding to 19 kavalactones, 3 dihydrochalcones and 8 minor components.[14] Ninety-six per

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cent of the organic extract derived from kava rootstock consists of six major kavalactones, methysticin, dihydromethysticin, kavain, dihydrokavain, demethoxyyangonin, and yangonin (Fig. 2).[14] Traditional kava preparations contain 0.3-20% kavalactones. Kavalactone and chalcone concentrations are increased when extracts are unfiltered.[13] Commercialised kava preparations extracted by organic solvents demonstrate a similar compositional ratio, excluding greater kavain and dihydrokavain content.[15]

Kava is commonly known for its anxiolytic relaxant effects and these are achieved by kavalactone ligation to the central nervous system (CNS) GABA receptors.[16] Modulation of GABA activity is mediated via alteration of lipid membrane structure, sodium channel function, MAO-B inhibition, and sodium and dopamine reuptake inhibition within the brain.[17] This disruption of the GABA cerebellar function induces impaired movement coordination and visual attention that accompanies Kava inebriation. Further side effects associated with excessive consumption are dryness and skin ulceration.[16]

In addition to its psychotropic effects, recent evidence has shown that some of the Kava components exert anti-cancer effects. There is growing interest about their potential impact on malignant cell proliferation, signalling, resistance to apoptosis, evasion of growth suppressors and angiogenesis. However, the potential anti-proliferative effects on oral squamous cell carcinoma (OSCC) has yet to be investigated. This systematic review aims to present the current literature surrounding Kava and its potential anti-cancer effects with a specific focus on OSCC.

Objectives

This qualitative systematic review aims to collate scientific evidence that constituents of Kava have anti-cancer properties and may be potential chemotherapeutic agents.

The specific questions addressed in this systematic review were:

1) Do the constituents of Kava have anti-proliferative effects on cancer cells *in vitro*?
2) Do the constituents of Kava have anti-proliferative effects on tumours in animal studies?
3) Do the constituents of Kava have cancer prevention properties?
4) What known anti-cancer pathways do the constituents of Kava interact with?
5) Which Kava constituents have the greatest anti-cancer potential?

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Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA Statement) was used for the data collection and sorting process. A table based on the PRISMA flow diagram is included (Fig. 3).

Literature search

On April 9, 2018, a comprehensive search of electronic databases OVID EMBASE, OVID MEDLINE and Web of Science was performed. An identical search strategy was employed across all databases.

The aim of the search was to find *in vivo* and *in vitro* experimental studies, allowing a summative evaluation of the preventive and treatment effects of Kava on cancer.

Search queries involved keyword searches for all cancer related terms and various Kava names including: anticancer OR anti-cancer OR anti-proliferative OR antiproliferative OR cancer OR carcinoma OR tumorigenesis OR neoplasm OR malignan* OR metastas* AND yaqona OR sakau OR rauschpfeffer OR kava OR kawa OR piper methysticum OR tudei OR piper wichmannii.

A second keyword search query was also performed for all cancer related terms and various active Kava constituents: anticancer OR anti-cancer OR anti-proliferative OR antiproliferative OR cancer OR carcinoma OR tumorigenesis OR neoplasm OR malignan* OR metastas* AND chalcones OR flavokawain OR flavokavain OR kavalactones OR desmethoxyyangonin OR 5,6-dehydroka* OR dihydromethysticin OR dihydrokavain OR dihydrokawain OR kavain OR kawain OR methysticin OR yangonin OR hydroxykavain OR hydroxykawain.

Selection criteria

No limits were placed on the search, and all languages were accepted. Papers that studied interactions between major kava constituents and cancer cells, *in vitro* or in animal models, were included. Technical reports were accepted. Studies using Kava constituents originating from other plant species were also included. No restriction was placed on what types of cancers were involved. Cell markers, cell proliferation/apoptosis and tumour sizes (*in vivo*) were common biomarkers. No restrictions were placed on the study design employed.

Studies were excluded if they were irrelevant to the cancer and Kava association. Papers studying the interaction of Kava or its constituents in non-cancer areas such as anxiety, hepatotoxicity or structure activity relationships were disregarded. However, the association

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between cancer and inflammation led to inclusion of papers discussing inflammatory effects of kava constituents if there was a concurrent focus on cancer. Studies of synthesised analogues of Kava constituents were not included, nor were studies solely focused on whole Kava extracts or fractions due to the large number of unknown variables that would be introduced. Case reports, letters, conference abstracts, reviews, epidemiological studies and retracted studies were excluded.

Data collection and sorting

The literature search identified a total of 2952 papers. Search results were imported into Endnote X8, compiled, and duplicates removed by the software, and then further scanned individually to remove manually undetected duplicates by two blind reviewers. A total of 1834 unique articles were found.

Utilizing the PRISMA protocol, 2 independent and blinded reviewers were enlisted to sort the relevance of articles by title.

Title screening

Out of the 1834 papers, 107 unique articles were selected with an inter-rater reliability (IRR) of 98.26%. If there was no accordance on one paper, it was evaluated by a third reviewer. A final total of 94 articles was unanimously selected for abstract screening.

Abstract screening

Of the 94 articles screened, a combined total of 55 articles were accepted (IRR: 92.55%). Disagreements were due to each reviewer's subjectivity. Discussion of the 7 differences resulted in a consensus of 3 articles being accepted. A final total of 51 articles was unanimously selected for full-text screening.

Full-text screening

4 reviewers screened the articles for data extraction to be included in the systematic review. Contentious articles were discussed by all reviewers. Unanimous agreement was required for the articles to be included in the final result.

Of the 51 articles screened, a combined total of 39 articles were accepted by the reviewers.

Data extracted from each article was recorded on the data collection table displayed as table 1.

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The following information was collected from each article: active kava molecules, model systems employed consisting of *in vitro* and *in vivo* models, experimental model as well as major findings incorporating anti-cancer mechanisms identified.

Risk of bias across studies

As with other bodies of scientific evidence, the potential effect of publication bias, favouring reporting of positive outcomes, cannot be excluded. Likewise, it is not known whether the authors reported only their most favourable results. Therefore, included studies underwent quality assessment according to QUIPS guidelines. Averaged across each risk of bias domain, we evaluated 85.9% percent of articles as having a low risk of bias, 11.54% of articles were identified to have a moderate risk of bias, and 2.56% were at a high risk of bias (Appendix 1).

Results

The initial search strategy identified 2952 potentially relevant citations of which, 1118 duplicates were removed. Following screening by title and abstract, 51 publications were included for full-text screening by 4 independent reviewers against the eligibility criteria. 12 publications were excluded and the remaining 39 were included for data extraction in the systematic review. Rationale for exclusion is presented in Appendix 2.

Of the 39 included studies, Flavokavain B (FKB) was the most studied constituent (21 articles); followed by Flavokavain A (FKA) (12 articles); dihydromethysticin (DHM) (7 articles) and desmethoxyyangonin (DMY) (4 articles); dihydrokavain and kavain (5 articles each); Flavokavain C (FKC) (4 studies); methysticin (4 articles); yangonin (3 articles); and yangonindimers (1 article). A single study was also conducted on the recently isolated yangonindimers. A complete list of the included studies are presented in Table 1.

Thirteen articles included animal models; these included tumour-inoculated and dietary NNK-induced mice models (A/J, TRAMP, C57BL/6, UPII-SV40T)[19 20,30,31,34,37-39,43], *in situ* human cancer tissue xenografted into SCID mice and nude mice and the Balb/C strain [15,32,49,50]; and the zebrafish model.[45] Of the 13 articles conducting animal studies, 7 also studied the effects of kava constituents on cancer cells *in vitro*.[15,19,20,31,32,49,50] Overall, 32 papers studied the effects of Kava constituents *in vitro*. Multiple cancer cell lines were studied, each representing diverse cancer types: breast, colorectal and lung cancer were commonly studied, while oral cancer was only studied in one publication.[27]

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Flavokavain B (FKB)

The most heavily researched constituent of Kava was found to be FKB. A total of 21 studies comprising 15 *in vitro* (including 2 *in situ*; 1 *ex vivo*), 1 *in vivo*, and 5 that assessed both models, examined the anti-cancer effects of FKB. Across these studies, there was unanimous agreement that FKB had anti-proliferative ability with the majority of papers reporting induction of G2/M arrest.[14,18,22,23,25,27,29,32,49,50,52,53] One paper suggested FKB induced G0/G1 arrest.[35] Additionally, there was a general consensus that FKB induced apoptosis.[14,18,20,22,23,25,27,29,38-40] Proposed mechanisms included increased expression of pro-apoptotic proteins. such as PUMA, Bim and Bax expression [28,29,35,36,38]; decreased expression of anti-apoptotic proteins, such as survivin and XIAP[33,35,36], ROS production[14,31,34]; or increase in caspase 3, 7, 8, and or 9.[28,36] Three studies suggested apoptosis may have been induced via PARP cleavage [27,29,33], although interestingly a single study reported that apoptosis was induced in the absence of PARP cleavage. [34] A single study, found that FKB did not induce apoptosis. [20] Other proposed anti-cancer effects of FKB included prevention of metastasis[19,30,32], anti-angiogenic effects [27,30,32], as well as regulation of immune and inflammatory functions.[19]

Flavokavain A (FKA)

A total of 12 studies comprising of 6 *in vitro* (including 1 *ex vivo*), 2 *in vivo*, and 4 that assessed both models examined the anti-cancer properties of FKA. FKA was found to induce apoptosis[18,21,41-43] and inhibit proliferation.[18,22,40-42] Proliferation inhibition was reported mainly through G2/M [21,42,43], and G1 cell cycle arrest [21], as well as c-myc inhibition.[18,40] Reported pathways inducing apoptosis included caspase activation[40,41], increase in pro-apoptotic protein expression such as Bim, Bax, DR5 and p27 [22,42], decrease in anti-apoptotic protein expression namely survivin and XIAP[22,40], and various mitochondrial membrane changes. [41] Liu et al. (2017) found evidence of synergism in apoptosis induction between FKA and Yangonin.[50] Other anti-cancer findings of FKA included the prevention of metastasis, inhibition of angiogenesis, reduction in inflammation, and enhanced immune function.[18,22,41] Solely, Johnson et al. (2011) concluded that FKA did not have significant anti-proliferative effects or apoptosis-inducing capabilities.[20]

Flavokavain C (FKC)

A total of 4 studies, 3 *in vitro* and 1 that assessed both *in vitro* and *in vivo*, examined the anti-cancer properties of FKC. There was a consistent suggestion that FKC inhibited cell

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proliferation in various cancer cell lines via G1 and G2/M arrest due to p21, p27, p53 upregulation.[40,44] Furthermore, FKC *in vitro* induced apoptosis via down regulation of apoptosis inhibitors XIAP, c-IAP1, c-IAP2, upregulation of pro-apoptotic signals, such as CHOP and GADD153, increasing ROS, and decreasing superoxide dismutase.[40,44,45] *In vivo*, FKC did not show anti-proliferative activity when administered alone to A/J mice.[20]

Dihydromethysticin (DHM)

A total of 7 studies including 3 *in vitro*[46-48] and 4 *in vivo*[23-26], examined the anti-cancer properties of DHM. All of the studies demonstrated that DHM had chemopreventative effects, with one study suggesting induction of apoptosis by G0/G1 arrest.[47] The remaining *in vitro* studies reported weaker anti-cancer effects of DHM.[46,48]

Methysticin

A total of 4 studies, 3 *in vitro* and 1 *in vivo*, examined the anti-cancer properties of methysticin. Methysticin has shown inhibition of NF-kB, a protein involved in cell survival, in A549 human lung adenocarcinoma, and on K562 chronic myelogenous leukaemia cell lines.[46,48] Methysticin resulted in downregulation of androgen receptor genes, but not actual androgen receptor mRNA expression in prostate cancer cell lines.[15] It was suggested methysticin's bioactivity is non-conformational,[46] and that its dioxymethylene group is essential to its activity.[48] All studies suggested methysticin may have promising anti-cancer activity, however the concentration tested and reported potency varied between studies.

Kavain

A total of 5 studies, 4 *in vitro* and 1 *in vivo*, examined the anti-cancer properties of kavain. Kavain was found to enhance growth inhibition of cancer cells, however with lesser potency than FKB and methysticin.[15,48] Minimal to no anti-proliferative or apoptotic effects of Kavain was observed on three different bladder cancer cells in contrast to similar doses of flavokavain A, B and C. [40] Finally, a single study found no effect of kavain on cell viability.[46]

Dihydrokavain

A total of 5 studies; 3 *in vivo* and 2 *in vitro*; examined the anti-cancer properties of dihydrokavain. The studies unanimously agreed that dihydrokavain showed no significant anti-cancer effects.[23-25,46,48]

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Desmethoxyyangonin (DMY) (also known as 5,6-dehydrokavain)

A total of 4 studies, 3 *in vitro* and 1 *in vivo*, examined the anti-cancer properties of DMY. DMY demonstrated a highly selective anti-proliferative effect in ovarian and lung cancer.[49] In contrast, one study found that DMY concentrations of 5–20 μg/ml did not have any effect on human squamous cell carcinoma of the cervix.[27] Finally, a study reported that DMY showed 100-300 times less NF-κB inhibitory activity than methysticin.[48]

Yangonin

A total of 3 studies, all *in vitro*, examined the anti-cancer properties of yangonin. Yangonin, was found to enhanced growth inhibition when combined with FKB, but demonstrated less potent growth inhibition independently.[15] In comparison to FKB, yangonin had weaker bioactivity in both prostate cancer and K562 human chronic myelogenous leukaemia cell lines.[15,46] Yangonin alone showed variable growth inhibition on murine cell lines as well as time and dose-dependent inhibition of human cell line growth.[50] When combined with docetaxel and FKA, yangonin synergistically induced autophagic cell death in bladder cancer cell lines.[50]

Yangonindimers

One *in vitro* study assessed yangonindimers, involving the combination of recently isolated dimers of yangonin and desmethoxyyangonin. The study showed no significant anti-proliferative activity on several human tumour cell lines.[51]

Oral Squamous Cell Carcinoma

Hseu et al. (2012) was the only identified study to investigate components of Kava in OSCC. The study assessed the chemopreventive effect of FKB on two human tongue oral squamous carcinoma cell lines, HSC-3 and Cal-27. Major anti-proliferative measures assessed included cell viability determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, cell cycle analysis by FACScan cytometer, and apoptosis by terminal deoxynucleotidyl transferase dUTP-fluorescein nick end-labeling (TUNEL) with fragmented DNA detection kit.[31] It was found that FKB significantly inhibited growth of HSC-3 cells and Cal-27. However, the effect was more pronounced in HSC-3 cells, where it caused accumulation of cells in G2/M phase.[31] Dose-dependent induction of apoptosis was also

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observed.[31]

Discussion

Kava has been of interest for its established anxiolytic effects. [52,53] However, there is now a growing recognition of its potential anti-cancer effects. This systematic review evaluates the current literature on the anti-cancer properties of Kava constituents, their possible mechanisms of action, and areas that require further investigation. This review was focused on individual constituents as opposed to extracts. This has allowed identification of molecules of greatest chemotherapeutic significance and comparison between papers.

The gathered publications strongly supported that multiple constituents of Kava possess significant anti-proliferative and apoptotic properties that warrant further research. Anti-cancer properties were mainly assessed by cytotoxicity evaluation through cell viability tests, such as MTT assays, with protein analyses used to assess potential mechanisms involved in apoptosis. Numerous studies also investigated the impact on proliferation regarding the possible cell cycle inhibition by Kava including stage of cell cycle arrest. Methodological heterogeneity limited direct result comparison between studies, although this may be reflective of the novel interest in the anti-cancer properties of Kava.

Chemoprevention refers to the administration of an agent intended to reduce or delay the initiation of carcinogenesis.[54] Substances demonstrating anti-proliferative or apoptosis-inducing mechanisms, such as constituents of Kava, could have potential as chemo-preventive therapeutics. Anti-proliferative properties were demonstrated in 32 out of the studied 39 papers. Of these papers, 27 presented *in vitro* assessment and 10 *in vivo*, which were mainly conducted in mice, and a zebrafish model[15,18-30].

Fifteen papers reported cell cycle arrest by FKA, FKB, and or FKC, at G2/M[14,21,27-29,31-38,42,43] or G1[21,39]. Cells accumulated in the G2/M phase undergo apoptosis through the Bax-initiated mitochondrial pathway.[33,38,42] Apoptosis was investigated in 24 out of 39 papers, 21 of which included *in vitro* components and 7 *in vivo* components. Apoptosis is characterised by key features, such as caspase activation, DNA fragmentation, and chromatin condensation.[31] Many of kava's active compounds instigate apoptosis through DNA fragmentation and mitochondrial membrane permeability via upregulation of the mitochondrial apoptosis cascade and Fas-mediated apoptosis of Caspase-8.[27,31,36] FKA, FKB and FKC induce apoptosis via the intrinsic pathway, associating with an increase in cytochrome c release leading to activation of Caspase 3, 7, 8 and 9.[14,27-29,31,33,36,37,40,41,44,45] Caspase activation has

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numerous effects, however the main mechanisms highlighted include PARP cleavage, increased ROS production, and consequent inhibition of PI3K/Akt phosphorylation in keratinocytes, reducing tumorigenesis.[14,27-29,31,33,34,44]

In addition to increased caspase activation, FKA and FKB were associated with increased expression of pro-apoptotic proteins and decreased expression of anti-apoptotic proteins. Specifically, there was an increase in Puma, Bim, and Bax, activating BAK and BAX proteins responsible for the initiation of apoptosis at the mitochondrial outer membrane.[28,29,35,36,38,42] There were further reports of increased DR5 expression and subsequent caspase 8 activation, and upregulation of p21, p27 and p53, all which play a role in gene inhibition/cell cycle arrest and anti-proliferative pathways.[21,27,35,43] FKA and FKB decreased the expression of anti-apoptotic proteins such as Bcl-2, BclX/L and Cdc2.[14,27,36,42] Exposure to flavokavains proposedly lead to down regulation of the inhibitor of apoptosis (IAP) protein family, allowing for progression of apoptosis along with decreased inflammation.[55] BclX/L prevents the release of mitochondrial contents, such as cytochrome C, whilst Cdc2 triggers entry into the M phase of the cell cycle and suppresses the action of Wee1.[42] [14,27,36] Wee1 typically slows admission into M phase to ensure adequate cell growth.[21,27,42] Thus, Kava compounds have the capability to both downregulate anti-apoptotic and upregulate pro-apoptotic proteins with the potential to influence numerous apoptotic pathways.

A number of studies investigated angiogenesis-inhibitory properties and found Kava compounds successfully inhibited vessel formation *in vivo* and *in vitro.*[18,22,30,32,41] Both FKA and FKB were shown to reduce angiogenesis in a dose-dependent manner.[18,22,27,30,32,41] For instance, FKA was found to downregulate the androgen receptor and subsequently alter angiogenesis in bladder tumour tissues.[22]

No studies reported the comparison of the anti-cancer capabilities of the constituents as a primary outcome. However, selected papers suggest that FKB may be the most potent constituent studied.[15] One study concluded that kavain, DHK, DHM and DMY are approximately 100-300 times less active than methysticin[48] whilst a further study commented on the lesser toxicity of FKA in comparison to FKB.[20,56]

Interestingly, a study undertaken by Johnson et al. (2011) on the effects of whole Kava extract in A/J mice found a statistically significant reduction in tumour multiplicity. However, they found that individual administration of FKA, FKB and FKC did not impact on adenoma multiplicity even at concentrations above that naturally occurring in Kava extracts.[20] Factors influencing these results could include variation in Kava extract, processing artefacts, strain of mice, tumour type and interactions of constituents and host

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ligands. This reveals the complexity of *in vivo* models and the need for further studies to translate *in vitro* findings.

Within the included publications, a total of 26 different cancers were assessed across the 32 studies solely on epithelial malignancies, 6 on mesenchymal malignancies and one study including both.[49] A single publication analysed the effect of the Kava constituent FKB in ectodermal-epithelial cancers, particularly oral squamous cell carcinoma and melanoma.[31] In contrast, the study of mesodermal-epithelial cancers and Kava constituents has been moderately established, particularly in colorectal and breast cancers.[14,18,19,34,41 42,44,45,49,51] Malignant epithelial lines studied may have potential mechanistic correlations in OSCC.

Mesenchymal cancer studies were the least investigated with primary focus on leukemia.[46 29] Consideration can be given however, to their potential application in the context of epithelial-mesenchymal transition in epithelial malignancy (EMT). The effect of Kava in sarcoma studies could reveal target pathways in invasive carcinomas, such as OSCC, demonstrating EMT with mesenchymal traits. For example, EMT has been implicated in metastasis and invasion by the PI3K/Akt pathways in OSCC.[57]

Conclusion

This systematic review found that particular constituents of Kava consistently displayed anti-cancer effects *in vitro* and *in vivo*. The current body of evidence primarily features the induction of apoptosis and cell cycle arrest in epithelial malignancy. These promising findings highlight an avenue for further research of Kava constituents in the prevention and treatment of OSCC. There remains a paucity of literature surrounding the chemotherapeutic potential of Kava in the field of oral oncology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legend

Figure 1: *Piper Methysticum* pressed plant specimens dated back to 1886, from the collection of the National herbarium of Victoria collection. Images were captured with a Leaf Aptus-II 10 Digital Back camera. Reproduced with permission from the Royal Botanic Gardens Victoria.

PRISMA flow chart of the systematic review

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Figure 2: Structures of the major kavalactones occurring in kava rhizome; (below) ground kava root.

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systematic review

Figure 3: PRISMA flow chart of the systematic review

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Ref.	Author, Year	Active molecule(s)	Model System	Experimental model	Major findings / mechanisms reported
[18]	Abu et al., 2016	Flavokavain B	Human breast cancer (MDA-MB231, MCF-7)	In vitro and ex vivo Cell culture, MTT assay, cell treatment, BrdU incorporation assay cell cycle analysis, annexin V/FITC assay, wound healing assay, transwell migration/ Invasion assay, HUVEC tube formation assay, ex vivo rat aortic ring assay, quantitative real-time PCR, western blot analysis, proteome profiler array	- Induced apoptosis in both MCF-7 and MDA-MB231; G2/M arrest was seen in MDA-MB231 cells. - Inhibited the in vitro migration and invasion in MDA-MB231 cells in a dose dependent manner - Inhibited angiogenesis; via suppressing the formation of vessels in HUVEC cells and within the rat aortic ring assay
[19]	Abu et al., 2015a	Flavokavain A	Mouse breast cancer (4T1) , Mouse lymphoma (YAC-1)	In vitro MTT assay, Wound healing assay, Transwell migration/ invasion assay In vivo TUNEL assay, tumor histopathology staining, immunophenotyping of splenocytes, splenocytes cytotoxicity assay, serum detection of IL-2 and IFN-γ levels, Nitric oxide detection assay, Quantitative real time PCR analysis, Western blot	- Dose dependant inhibition of proliferation of 4T1 cells - Anti metastatic effects in vitro - Decreased volume and weight of tumors in vivo - Induced apoptosis in vivo - Suppresses proto oncogene C-myc - Enhanced T cell immunity via upregulation of interleukin 2 and interferon gamma, resulting in an increase Th, Tc, NK cell populations - Anti inflammatory effect via reduction in COX-2 expression

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[20]	Abu et al., 2015b	Flavokavain B	Mouse breast cancer (4T1)	In vitro MTT analysis, cell cycle analysis, wound healing analysis, in vitro migration/ invasion assay, animal and diet, tumour inoculation and treatment, terminal deoxynucelotidyl transferase dUTP nick end labeling analysis of the tumours, haematoxycilin and eosin histology staining, nitric oxide detection, lung, liver and spleen clonogenic assay, immunophenotyping, serum detection, bone marrow smearing, total red blood cell count, real-time quantitative polymerase chain reaction, western blot analysis, proteome profiler mouse angiogenesis, TUNEL analysis	- Inhibited proliferation and induced apoptosis of 4T1 cells - Inhibition of migration and invasion of 4T1 cells - Regulates several immune system markers - Possesses antimetastatic abilities - Regulates inflammation and metastasis-related genes and proteins
[21]	Abu et al., 2014	Flavokavain A	Human breast cancer (MCF-7, MDA-MB231)	In vitro MTT assay, BrdU assay, Annexin V analysis, cell cycle analysis, JC-1 mitochondrial dye, AO/PI dual staining, caspase 8/9 fluorometric assay, PCR, Western blot In vitro and ex vivo Scratch assay, transwell migration/invasion assay, HUVEC tube formation assay, ex vivo rat aortic ring assay, quantitative PCR, Western blot	- Dose-dependently inhibits MCF-7 and MDA-MB231 cell proliferation - Cell-cycle accumulation at the G2/M phase in MDA-MB231 - Dose dependently induced apoptosis in MDA-MB231 and MCF-7 cells through mediation of caspase 8 and 9, and regulation of several apoptosis genes and proteins - Induced changes in mitochondrial membrane potential. Higher doses reduces membrane polarisation - Inhibited motility and invasiveness of

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					MDA-MB231 cells in vitro - Dose-dependently reduced angiogenesis
[22]	An et al., 2012	Flavokavain B	Human lung cancer (H460), primary mouse embryo fibroblasts (MEF) deficient for Bax (double knockout)	In vitro MTT assay, cell morphology observation and DAPI staining, fluorescence-activated cell sorting analysis of cell cycle distribution, Western blot analysis, plasmid transfection	- Inhibited cell proliferation - Induced G2-M cell cycle arrest - Induced apoptosis; through Bax initiated mitochondrial pathway, significantly decreased the levels of survivin and X-linked inhibitor of apoptosis (XIAP), cytochrome c release and activated the cleavage of PARP, caspase-7, and caspase-9 - Disruption of Bax almost completely impaired effect of FKB-induced growth inhibition (MEFs) - Activated the stress-responsive mitogen activated protein kinases (MAPKs) signalling
[23]	Chang et al., 2017	Flavokavain B	Human gastric carcinoma (AGS, NCI-N87, Kato-III, TSGH9201), primary mouse hepatocytes, normal stomach and intestinal cells (Hs738), athymic nude mice (BALB/c-nu) with AGS tumour cell inoculation	In vitro Cell culture, drug treatment, MTT assay, colony formation assay, measurement of ROS generation, cell-cycle analysis, western blot analysis, GFP-LC3 plasmid transfection and GFP-LC3 dot formation In vivo Acridine orange staining, transfection of shRNA	- FKB is potently cytotoxic to human gastric cancer cells, mildly toxic towards normal (Hs738) cells and primary mouse hepatocytes; induced AGS cell death characterized by autophagy as evidenced by increased LC3-II accumulation, GFP-LC3 puncta and acidic vesicular organelles (AVOs) formation, without resulting procaspase-3/ PARP cleavage

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				targeting LC3, Bax transfection, apoptotic DNA fragmentation, tumour cell inoculation animals, histopathological analyses of xenografted tumour, western blotting of xenografted tumours, animal survival study	- FKB induced apoptosis via ROS generation and ROS inhibition; indicated by ROS-mediated autophagy in AGS cells - FKB induces G2/ M arrest and alters cell-cycle proteins; ROS-JNK signaling - FKB inhibits apoptotic Bax expression, and Bax-transfected AGS cells exhibit both apoptosis and autophagy; thus, FKB-inactivated Bax
[24]	Dai et al., 2015	Dihydromethysticin	Human osteosarcoma (MG-63)	In vitro MTT assay, computer-assisted phase, contrast microscopy, annexin V-FITC assay, flow cytometry, mitochondrial membrane potential measurement, western blot analysis	- Dose dependently inhibited growth of MG-63 cells via chromatin condensation. - Induced both cytotoxic and cytostatic effects resulting in marked vacuolization processes and ultimately cell death - The percentage of cells in G2/M phase decreased considerably with increase in DHM dose whilst fraction of G0/G1 cells increased significantly with increasing concentration of DHM - The number of cells with depolarized mitochondria increased with DHM dose
[25]	Eskander et al., 2012	Flavokavain B	Human uterine leimyosarcoma (SK-LMS-1), human endometrial	In vitro MTT assay, Fluorescence activated cell sorting analysis of apoptosis, Fluorescence activated cell	- Selective inhibition of growth, greater effect in SK-LMS-1 and ECC-1 than in T-HESC

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			adenocarcinoma (ECC-1), human normal endometrial fibroblasts (T-HESC)	sorting analysis of cell cycle, Western blot analysis, Real-time reverse transcription-polymerase chain reaction	- Induced G2/M arrest and apoptosis via upregulation of DR5, puma and bim expression, downregulation of apoptosis inhibitor survivin
[26]	Folmer et al., 2006	Kavain, Flavokavain A, Flavokavain B, Methysticin, Dihydromethysticin, Yangonin, Dihydrokavain, Demethoxyyangonin	Human chronic myelogenous leukemia (K562), Human T cell leukemia (Jurkat)	In vitro Transient transfection, luciferase reporter gene assay, Electrophoretic mobility shift assay (EMSA), Western blot analysis, Protein Kinase assays	- Yangonin has weak bioactivity suggesting methoxy group of A ring hinders its activity - compounds with aromatic rings solely substituted with a methoxy group tend to lack anticancer activity. - FKA inhibits MAPKAP-K3 - could be responsible for apoptotic mechanisms induced by Kava.
[27]	Hseu et al., 2012	Flavokavain B	Human oral squamous carcinoma (HSC-3), human melanoma (A-2058), oral adenosquamous carcinoma (Cal-27), human lung carcinoma (A-549)	In vitro MTT colorimetric assay, Flow cytometric analysis, TUNEL assay, Fluorescent imaging of mitochondria and endoplasmic reticulum, Immunofluorescence assay, Fluorescence microscopy for apoptosis, Western blot	- Significantly reduced HSC-3, A-2058, Cal-27 cell survival in dose-dependent manner, minimal effect against A-549. Time- and dose-dependent for HSC-3 - Promotes growth inhibition by G2/M phase arrest; inhibits cell cycle progression by reducing levels of cyclin A, cyclin B1, Cdc2, and Cdc25C (HSC-3) - Induces apoptosis (HSC-3); induces Apoptotic DNA Fragmentation (HSC-3), induces mitochondrial membrane permeability (HSC-3), upregulates Mitochondrial Apoptotic

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					Cascades (HSC-3), activates Fas-Mediated Apoptosis through the activation of Caspase-8 - Downregulates p38 MAPK and Upregulates ERK and JNK Proteins (critical in cell fate, Role in G2/M Arrest and Apoptosis, HSC-3) - Induces Intracellular ROS Generation (HSC-3) - Inhibits Phosphorylation of PI3K/Akt in HSC-3 (dysregulation of the PI3K/Akt signaling pathway leads to tumorigenesis in vitro and in vivo)
[28]	Jandial et al., 2017	Flavokavain A	Human breast adenocarcinoma (SKBR3), human breast cancer (MCF7/HER2)	In vitro MTT assay, Soft agar colony formation, Flow cytometric analysis of cell cycle distribution, Western blotting analysis, In vitro kinase assay, DAPI nuclear staining	- G2M arrest and apoptosis induction via downregulation of HER-2 gene that is known for suppressing proapoptotic proteins, increased expression of proapoptotic proteins Bim and BAX, decreased expression of anti apoptotic proteins Bcl_2, BclX/L, XIAP, survivin, dephosphorylation of Cdc25C, downregulation of Cdc2 hence Myt1 and Wee1 expression
[29]	Ji et al., 2013	Flavokavain B	Human osteosarcoma (OS160, 143B , SaOS-2, MG-63, U2OS), Murine	In vitro MTT assay, Soft agar colony formation assay, DAPI staining for apoptotic cell nuclei, Caspase	- Inhibits proliferation of osteosarcoma cells - Induces apoptosis (143B, SaOS-2) through increased expression of Fas, Puma and Bax,

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			bone marrow cells (derived from Balb/c), Human normal small intestine epithelial cells (FHS)	activity assay, Fluorescence-activated cell sorting (FACS) analysis, Protein isolation and Western blot analysis, Zymogram assay, Motility and invasion assay	while down-regulating the expression of Bcl-2 and Survivin, increases Caspase 8, 9, 3/7 activity (143B, SaOS-2) - Suppressed in vitro motility (143B) and invasiveness (143B, SaOS-2); Inhibited MMP-2 and MMP-9 secretion (143B) - Induces G2/M arrest (143B, SaOS-2 cells); Caused significant decrease in Cyclin B1, Cdc 25c and increase in p-Cdc2 in a time-dependent manner, increase in Myt1 not time dependent (143B) - No significant growth inhibitory effects on murine bone marrow cells - Significant differences in cell viability for 143B and FHS, both reducing with FKB
[30]	Johnson et al., 2011	Kava extract Flavokavain A, Flavokavain B, Flavokavain C	A/J mice with dietary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo(a)pyrene (BaP)–induced lung tumorigenesis	In vivo Tumour count by dissecting microscope, detection of serum enzymatic levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin, Western Blot analysis, immunohistochemistry staining; H&E staining	- Kava extract dose dependently reduced lung adenoma multiplicity - FKB significantly decreases adenoma multiplicity but at much higher concentration than found in kava; FKA and FKC not significant effect - No significant change to diet consumption, ≤6% weight loss for highest kava concentration, No significant change to liver weight and serum enzyme levels

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					- No induction of apoptosis (no PARP cleavage) - Dose dependent decrease in PCNA protein
[14]	Kuo et al., 2010	Flavokavain B	Human wild type and p53−/− colorectal carcinoma (HCT116), human lung carcinoma (A-549), mouse fibroblast (NIH3T3), human fibroblast (HFW)	In vitro Colony formation assay, Apoptosis assays, Confocal microscopy, Cytofluorimetric analysis of mitochondrial membrane potential, Measurement of intracellular calcium concentrations, Measurement of ROS, Cell cycle analysis, Western blot analysis, Real-time reverse transcription - polymerase chain reaction	- Concentration and time dependant cytotoxic effect - Induction of G2/M accumulation, autophagy, and the intrinsic apoptosis pathway via the initiation of ROS-mediated apoptosis, GADD153 up-regulation resulting in ER stress response and downregulation of antiapoptotic Bcl-2 expression, induction of phosphorylation of p38 MAPK, and induction of cytochrome c release and Bak translocation resulting in mitochondria dependant apoptosis
[31]	Li et al., 2015	Flavokavain A	Human prostate adenocarcinoma (DU145, PC3, 22Rv1), prostate stromal cells (PrSCs), TRAMP mice (C57BL)	In vivo Western blot analysis, Plasmid and siRNA transfection, TUNEL assay In vitro MTT assay, NEDD8 conjugation assay, molecular docking, western blot analysis	- Induced G2-M arrest through activation of Myt1, Wee1, and CDK1 - Induced G1 arrest via decreasing cyclin-dependent kinase-2 (CDK2) kinase activity, increasing p21/WAF1 and p27/ KIP1 expression, reducing CDK1-inhibitory kinase expression - Degraded SKP2; a protein associated with tumor development, via induction of proteasome dependent degradation

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[15]	Li et al., 2012	Kavain, 5',6'-dehydrokavain, yangonin, methysticin, Flavokavain B Kava root extract	Human prostate cancer (LNCaP, LAPC-4, 22Rv1, PC-3, DU145, C4-2B) Human prostate normal (WPMY-1) Tumour xenograft in SCID mice (GM0308, RC0309)	In vitro MTT Assay, Western Blot analysis, Quantitative RT-PCR, Transfection, promoter activity and luciferase assay, Chromatin immunoprecipitation In situ Serum prostate-specific antigen, Caliper measurement of tumor size Immunohistochemistry staining	- Inhibits growth of AR-expressing PCa cells; Kava root extract more potent than kavalactones (K, Y, 5'6-DHK, M) but less potent than FKB - Decreases expression of AR target genes PSA and TMPRSS2 through acceleration of AR protein degradation (Kava root extract and kavalactones) - FKB down-regulates expression of AR and its target genes (PSA and TMPRSS2) - Kavalactones (K, Y, 5'6-DHK, M) and FKB combination results in enhanced inhibitory effect on growth of C4-2B cells and expression of AR protein - Kava extract and FKB decrease growth of patient-derived PCa xenografts in SCID mice, AR expression in tumor tissues, serum PSA levels
[32]	Lin et al., 2012	Flavokavain B, Desmethoxyyangonin	Human squamous carcinoma from glandular cancer of cervix (KB), human gingival fibroblast (HGF) Tumour xenograft in nude mice	In vitro Terminal deoxynucleotidyl transferase-meditated dUTP nick end-labeling assay for DNA apoptotic fragmentation, Flow cytometric analysis (incl. Cellular DNA content, Mitochondrial membrane potential), Fluorescence microscopy/flow cytometry measuring ROS generation, Western	- FKB induced cell death (viability or growth) in a dose- and time-dependent manner - DMY concentrations of 5–20 μg/ml did not affect KB cells at 24h - FKB cytotoxic to HGF above 30μg/ml at 24h

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				Blotting, Zymography (MMP-9 activity), In situ Apoptosis detection by TUNEL with the Klenow DNA fragmentation detection	- FKB induced apoptotic DNA fragmentation, release of cytochrome c, activation of caspase-3 and -9 and cleavage of PARP, activation of the Fas-mediated apoptosis pathway by FKB results in activation of caspase-8 and cleavage of Bid, induces dysregulation of Bcl-2 and Bax protein, induced ROS generation and mitochondrial dysfunction - Sub-G1 accumulation and G2/M arrest in FKB-treated KB cells; dose- and time-dependent reductions in mitotic cyclins A and B1, mitotic-cyclin-dependent kinase Cdc2 and mitotic phosphatase Cdc25C expression, increases expression of p21/WAF1, Wee1 and p53 - FKB dose-dependently affects metastasis-related protein expression; reduction of MMP-9 and u-PA expression, upregulation expression of specific endogenous inhibitors TIMP-1 and PAI-1 - FKB In vivo inhibition of KB xenograft growth (tumour volume); apoptotic DNA fragmentation in xenograft tumors
[33]	Liu et al.,	Flavokavain A,	Human bladder cancer (T24,	In vitro	- Yangonin and 5′ 6'-dehydrokavain are

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	2017	yangonin, 5′ 6'-dehydrokavain	RT4, UMUC3, HT1376, HT1197), mouse embryonic fibroblasts (MEFs)	MTT assay, colony formation assay, western blot analysis, 7-Methyl-guanosine cap binding assay, stable transfection, fluorescence microscopy, electron microscopy	potent inducers of autophagic cell death in bladder cancer cells - Yangonin induces autophagy via increased expression of beclin and ATG5. - Yangonin reduces the viability of bladder cancer cell lines, and acts synergistically with apoptosis-inducing agents such as docetaxel and flavokavain A; growth inhibitory effects of yangonin were attenuated in TSC1 or LKB1 knockout mouse embryonic fibroblasts, suggesting that TSC1 and LKB1 expression may contribute to optimal growth inhibition by yangonin
[34]	Liu et al., 2013	Flavokavain A	Transgenic mouse model (UPII-SV40T)	In vivo Immunohistochemistry, DeadEnd colorimetric TUNEL assay, Western blotting	- Concentration and time dependant anti tumor growth effect in vivo - Induced apoptosis via upregulation of proapoptotic protein DR5, p27 and TUNEL-positive apoptotic cells, downregulation of antiapoptotic proteins Ki67, survivin, and XIAP, downregulation of androgen receptor thus altering angiogenesis in bladder tumor tissues - Selective, no effect on organ:body weight change, weight loss, or food / water consumption

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[35]	Malami et al., 2017	Crude extracts FKB Alpinetin compounds (APN)	Human colon cancer cells (HT-29), human hepatic progenitor cells (HepaRG), Monkey fibroblast-like kidney cells (Vero)	In vitro Cell Viability study (MTT assay), RT-PCRof mRNA expression, Fluoresence imaging of 18S RNA expression, Cell Cycle analysis, DNA Fragmentation analysis, Extraction of total protein, Western blot analysis	- UCK2 mRNA expression significantly downregulated in all HT-29 cells treated with FKB and APN - consequent reduced 18s rRNA in HT-29 cells. - G0/G1 phase inhibition by FKB - FKB and APN induced p53 dependent apoptosis. - MDM2 protein expression significantly downregulated with increased p53 protein expression in a time-dependant manner following FKB and APN treatment - essential in triggering cell cycle arrest and apoptosis in HT-29 cells
[36]	Mustahil et al., 2013	5,6-dehydrokavain, flavokavain B, pinostrobin, pinocembrin, beta-sitosterol	Human lymphoblastoid cancer (T4 cells)	In vitro Cytotoxic assay, antimicrobial assay, DPPH radical scavenging activity assay	- All crude extracts of the plant (5,6-DHK, FKB, pinostrobin and pinocembrin together with Beta-sitosterol) demonstrated strong cytotoxicity against CEMss (T4) cancer cells - FKB was the most cytotoxic isolate; most crude extracts and isolated compounds showed weak activity in antimicrobial and diphenylpicryllhydrazyl (DPPH) radical scavenging activity tests
[37]	Narayanapillai et al, 2014	Dihydromethysticin Dihydrokavain Desmethoxyyangonin	A/J mice with dietary 4-(methylnitrosamino)-1(3-pyridyl)-1-butanone (NNK)	In vivo DNA isolation following Genomic-tip 100/G protocol, Quantification of DNA adducts in the	- DHM and methysticin significantly reduced NNK-induced O 6-mG while other compounds did not, importance of

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		, kavain, methysticin		lung tissues by LC-ESI-MS/MS, H&E staining	methylenedioxy functional group highlighted, minimum chemopreventive dose of DHM 0.01-0.1mg/g dietary - DHM significantly reduced NNK-induced adenoma multiplicity
[38]	Narayanapillai et al, 2016a	Dihydromethysticin Dihydrokavain	Female mice with NNK induced lung adenoma (C57BL/6)	In vivo Quantification of O6-mG adduct in lung tissues, Urinary NNAL-O-Gluc and free NNAL quantificatoin, western blotting analysis, quantitative reverse transcription polymerase chain reaction	- DHM presented chemopreventive effects; DHM reduced the level of NNK and NNAL induced DNA damage in lung tissue but not liver tissue, and dose dependently reduced carcinogen (O6-mg) levels - Suggests chemopreventive effect independent of Ahr pathway - DHK had no effect
[39]	Narayanapillai et al., 2016b	Dihydromethystycin, Dihydrokavain, Kava extract	A/J mice with dietary 4-(methylnitrosamino)-1(3-pyridyl)-1-butanone(NNK)	In vivo CYP2A5 Enzymatic Assays, Urine Processing to Convert NNAL-gluc into NNAL and Liquid Chromatography with Tandem Mass Spectrometry Quantification, Direct LC-MS/MS Detection and Quantification of NNAL-O-gluc, NNK, and NNAL in Urine and Serum Samples, NNAL Glucuronidation Activity of Mouse Lung and Liver Microsomes	- DHM and kava had no effect on NNAL formation from NNK - Unlikely that the DHM primary mode of action is inhibition of CYP2A5-mediated NNAL bioactivation - Higher amounts of NNAL-O-gluc detected in DHM-treated mice compared to control - Enhanced NNAL glucuronidation activity in lung and liver tissues upon dietary DHM treatment - DHK had no observable effect.

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[40]	Phang et al., 2017	Flavokavain C	Human colon adenocarcinoma (H-29), human colorectal carcinoma (HCT 116)	In vitro Annexin V-FITC and PI assay, DNA Fragmentation detection, Measurement of mitochondrial membrane potential, Measurement of intracellular ROS level, Superoxide dismutase inhibiton activity, Measurement of caspase -3,-8 and -9, Cell cycle analysis, Western blot	- Induced reduction in cell proliferation - Induced G1 and G2-M arrest via p21, p27, p53 upregulation - Induced apoptosis via disruption of mitochondrial membrane potential activating caspases and PARP cleavage, downregulating apoptosis inhibitors XIAP, c-IAP1, c-IAP2, upregulating apoptotic signal GADD153 via ER stress pathway, increased ROS, and decreased SOD
[41]	Phang et al., 2016	Flavokavain C	Colon carcinoma cells (HCT 116), human colon adenocarcinoma (HT-29), human lung carcinoma (A549), human cervical carcinoma (CaSki), human breast adenocarcinoma (MCF7), non-cancerous human colon fibroblast (CCD-18Co)	In vitro Cytotoxic assay, Morphological assessment of cell death by phase contrast and fluorescence microscopy, Plasma membrane, alteration analysis, Assessment of changes in mitochondrial membrane potential, Detection of DNA fragmentation by TUNEL assay Assay for activation of Caspase-3/8/9, Cell Cycle analysis using PI staining and flow cytometry, Western blot analysis - analysis of apoptosis related proteins in HCT116 following FKC treatment	- Reversing functional groups in FKC resulted in pronounced cytotoxic activity of FKC in HCT 116 cells, cytotoxic and apoptotic activities of chalcones are dependent on its molecular structure. - Gradual increase in cytosolic AIF and Smac/DIABLO concentration with FKC treatment. (AIF is involved in induction of caspase-independent chromatin condensation and DNA fragmentation). - Higher amounts of caspase-8 detected in comparison to active caspase-9 in HCT116 cells with FKC treatment (dose-dependent). (Caspase 8 activation inhibits apoptosis triggered by death receptors.)

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					- Increase in CHOP levels following FKC treatment. (CHOP is a key factor in ER stress-induced apoptosis via outer mitochondrial membrane permeabilization, caspase activation, and amplification of death signals) - Suggested that an interplay between Akt signalling and MAPK apoptosis pathways and cell cycle arrest in HCT116 cells by FKC. FKC inhibited Akt activation, resulting in increased ERK1/2 phosphorylation. FKC activated p38 MAPK to some extent.
[42]	Pinner et al., 2016	Flavokavain A, Flavokavain B	Human hepatocellular carcinoma (HepG2)	In vitro RNA collection and real-time RT-PCR, Protein collection and Western blots, Total GSH assay, fluorescent viability	- FKA is considerably less toxic than FKB to HepG cells - FKA and FKB increase total GSH levels - FKA and FKB induce antioxidant and heat shock gene expression; FKA protection against H2O2 greater than FKB
[43]	Puppala et al., 2017	Dihydromethysticin	Mouse lung tumorigenesis (A/J)	In vivo CO2 dosing, DNA isolation, LC-MS/MS quantification, potassium permanganate solution staining, analytical thin layer chromatography, compound visualisation via UV light, mass spectrometry	- Methylenedioxy and lactone functional groups on the chemopreventive activity of DHM used three rationally designed synthetic analogs that can respectively block methylene hydroxylation, lactone hydrolysis, or both routes of metabolism; methylenedioxy functional group of DHM is

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					critical for its chemopreventive activity while the lactone functional group tolerates modifications - Compounds 13 and 15 , devoid of the dioxy functional group of DHM while retaining the five-membered ring, did not show any significant inhibitory activity against NNK-induced O6 -mG formation or lung tumor multiplicity in A/J mice; 14 , with the intact methylenedioxy functional group but the mask of the lactone functional group, recapitulated the O6 -mG adduct reduction potential and antitumorigenic efficiency of DHM. The reduction in O6-mG correlates well with the blockage of lung adenoma formation - DHK, which lacks the methylenedioxy group, is inactive against NNK-induced DNA adducts and tumorigenesis
[44]	Roman Junior et al., 2017	5,6-dehydrokavain (Desmethoxyyangonin), Dihydro-5,6-dehydrokavain	Human glioma (U251), human breast adenocarcinoma (MCF-7), human ovarian cell expressing drugs resistance	In vitro Antiproliferative assays	- 5,6-DHK demonstrates highly selective antiproliferative effect

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			(NCI-ADR/RES), human renal cell adenocarcinoma (786-o), human large cell lung cancer (NCI-H460), human prostate adenocarcinoma (PC-3), human ovary adenocarcinoma (OVCAR-03), human colon adenocarcinoma (HT-29), human chronic myeloid leukemia (K-562), immortalized human keratinocytes (HaCat)		
45	Rossette et al., 2017	Flavokavain B	Human umbilical vein endothelial cells (HUVECs), human brain endothelial cells. Zebrafish model	In vitro Cell viability MTT assay Tube formation assay Wound healing assay In vivo Zebrafish strain and drug treatment	- Antimetastatic action of FKB in vivo might be related to inhibition of angiogenesis. - Significant inhibition of cell migration at low FKB concentration. - Dose-dependently reduced endothelial cell differentiation into capillary structures - Dose dependently inhibited HUVEC tube formation - Zebrafish model demonstrated dose-dependent reduction of subintestinal veins

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					and intersegmental vessel formation as well as disruption of vascular morphology of SIVs.
[46]	Seo & Oh, 2013	Flavokavain B	Non-small Cell Lung Cancer (H1975)	In vitro Proliferation assay, Western Blot	- Dose and time dependently Inhibited cell proliferation - Robust degradation of Hsp90's client proteins including EGFR, Met, Her2, Akt, and Cdk4 in a concentration-dependent manner
[47]	Shaik et al., 2009	Methysticin, Kavain, Dihydrokavain, Dihydromethysticin, Desmethoxyyangonin	Human lung adenocarcinoma (A549)	In vitro Luciferase-based assay, silica gel chromatography, HPLC, H NMR, C NMR, optical rotations, mass spectrometry analysis, western blot analysis	- Kavain, dihydrokavain, DHM, and desmethoxyyangonin are about 100–300 times less active than methysticin
[48]	Song et al., 2017	Yangonindimer A, B and C	Human lung cancer cell line (NCI-H46), human colorectal adenocarcinoma (SW480), human hepatocellular carcinoma (HepG2)	In vitro MTT assay	- None of the compounds showed significant cellular proliferation inhibition
[49]	Tang et al., 2015	Flavokavain B	Human normal renal cells (HK-2), human acute	In vitro Cell viability assay, Apoptosis assay	- Inhibited proliferation in vivo and in vitro - Dose dependently induced intrinsic

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			lymphoblastic leukemia (CEM-C1), human acute lymphoblastic leukemia (RS4-11), Balb/c xenograft mice (CCRF-CEM), human acute lymphoblastic leukemia (B-ALL or T-ALL)	Western blot assay In vivo Ex vivo Isolation of bone marrow specimens from patients	apoptotic pathway through cleavage and activation of caspase 3, and cleavage and degradation of PARP - Induced transcription dependent pathway of apoptosis in vitro and in vivo through increasing p53 expression, resulting in the upregulation of pro apoptotic genes Puma and Bax in vivo and in vitro - Dose dependant inhibition of proliferation observed in B-ALL and T-ALL but no effect on normal cells ex vivo
[50]	Tang et al., 2010	Flavokavain A, Flavokavain B	In vitro Human prostate cancer (LNCaP, LAPC-4, DU145 and PC-3), Normal prostate epithelial (PrECs) and stromal cells (PrSCs) NCR-nu/nu (nude) mice DU145 xenograft model	In vitro MTT assay, Fluorescence-activated cell sorting analysis of apoptosis, Quantification of apoptosis by ELISA, RT-PCR, RNA interference In vivo Western blot, Immunoprecipitation	- FKB inhibits growth of AR-negative, hormone refractory PCa cell lines (DU145, PC3) ~ 90%, partially reduces growth of AR-positive, hormone sensitive PCa cell lines (LNCaP, LAPC4) ~ 32-50%; FKB most potent of flavokavains - Minimal effects on normal primary prostate epithelial and stromal cells - FKB induced apoptosis; activation of caspase-3,-8 and -9 activities in AR-negative PCa, causes PARP cleavage (DU145 and PC-3), increases protein and mRNA expression of death receptor 5 (DR5) and

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					enhances TRAIL ligand induced apoptosis, activates Bax and mitochondrial-mediated apoptotic pathway by upregulation of Bim and Puma and downregulation of XIAP and survivin - FKB inhibits tumor growth in vivo in a DU145 xenograft model and induces Bim expression in tumor tissues
[51]	Tang et al., 2008	Flavokavain A	Human bladder cancer (T24, UMUC3, TCCSUP, 5637, HT1376, HT1197)	In vitro MTT assay, fluorescence-activated cell sorting analysis of cell cycle distribution, western blot analysis, P27 and p21 Degradation assay, real-time reverse transcription - PCR Immunoprecipitation and kinase assay, SiRNA suppression of p53	- Induced G2-M arrest via suppression of p53 expression by small interfering siRNA in RT4 cells restored Cdc25C expression and down-regulation of p21/WAF1 expression, which allowed Cdc25C and CDK1 activation, which then led to a G2-M arrest and an enhanced growth-inhibitory effect by FKA - Selectivity of flavokavain A for inducing a G2-M arrest in p53-defective cells; FKA caused a pronounced CDK1 activation and G2-M arresting p53 knockout but not in p53 wild-type HCT116 cells
[52]	Yeap et al., 2017	Flavokavain B	Human Cervical cancer (HeLa)	In vitro MTT assay, Flow Cytometry, Gene Expression Profiling Using Microarray, qRT-PCR, Proteome Profiler Antibody Human Cell Stress Array,	- Cytotoxicity (HeLa) - Induced G2/M phase arrest - Induced apoptosis; loss of membrane potential, differentially regulated mRNA

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				Superoxide Dismutase (SOD) and Glutathione (GSH) Quantification	expression of cell cycle, apoptosis, cell stress, and MAPK pathways - Upregulation of MAPK pathway, GPx3, HMOX1, CAT from the oxidative pathway, and DDIT3 from the apoptosis pathway, cytochrome C, phospho-p38 alpha (T180/Y182), SOD2, phospho-HSP27 (S78/S82), and HSP70 change - Protects HeLa cells From H_2O_2-induced cell death, activation of antioxidant neutralizes H_2O_2-induced ROS, enhanced GSH and SOD Levels
[53]	Zhao et al., 2011	Flavokavain B	Human oral adenoid cystic carcinoma (ACC-2)	In vitro MTT assay Cell morphology observation and DAPI staining Fluorescence-activated cell sorting analysis of cell cycle distribution Measurement of cytochrome c release from mitochondria RNA interference Western blot analysis RNA isolation and quantitative real-time RT-PCR	- FKB could significantly inhibit the cell proliferation of oral adenoid cystic carcinoma ACC-2 cells; Induced cell-cycle G2-M arrests in ACC-2 cells and subsequent apoptosis in the mitochondrial apoptotic pathway - Induced mRNA expression of Bim, Bak, bax, Bcl-2; Bim acted as a potential transcriptional target for FKB
[54]	Zi &	Flavokavain A, B,	Human bladder cancer (T24,	In vitro	- Kava extract and FKA, FKB, and FKC

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	Simoneau, 2005	and C, kavain	RT4, EJ), NCR-nu/nu (nude) mice	Cell viability 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, flow cytometry assays, measurement of cytochrome c release from mitochondria, western blotting and immunoprecipitation, soft agar colony formation In vivo	cause strong antiproliferative and apoptotic effects in human bladder cancer cells; FKA results in cleavage of caspase-3/9 and poly (ADP-ribose) polymerase in T24 cells in a dose- and time-dependent manner. The loss of mitochondrial membrane potential and release of cytochrome c caused by FKA are associated with an increase in Bax/Bcl-2 ratio and Bax confirmation change in T24 cells - FKA decreases the levels of X-linked inhibitor of apoptosis and surviving T24 cells - FKA inhibits anchorage-independent growth of EJ cells in soft agar and tumour growth in nude mice

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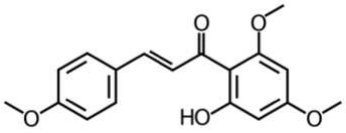
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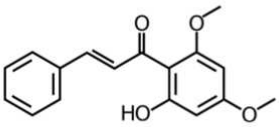
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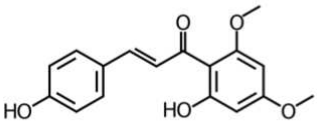
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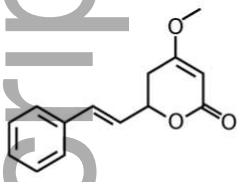
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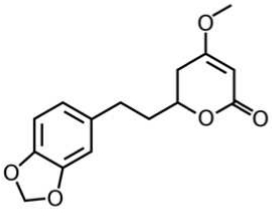
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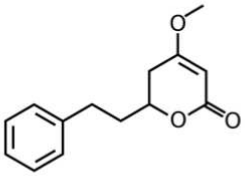
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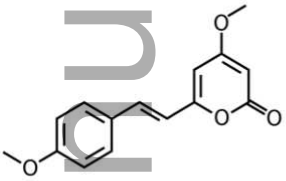
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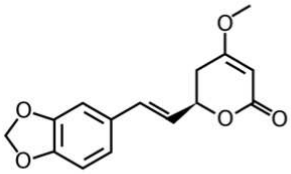
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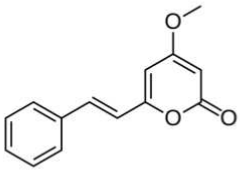
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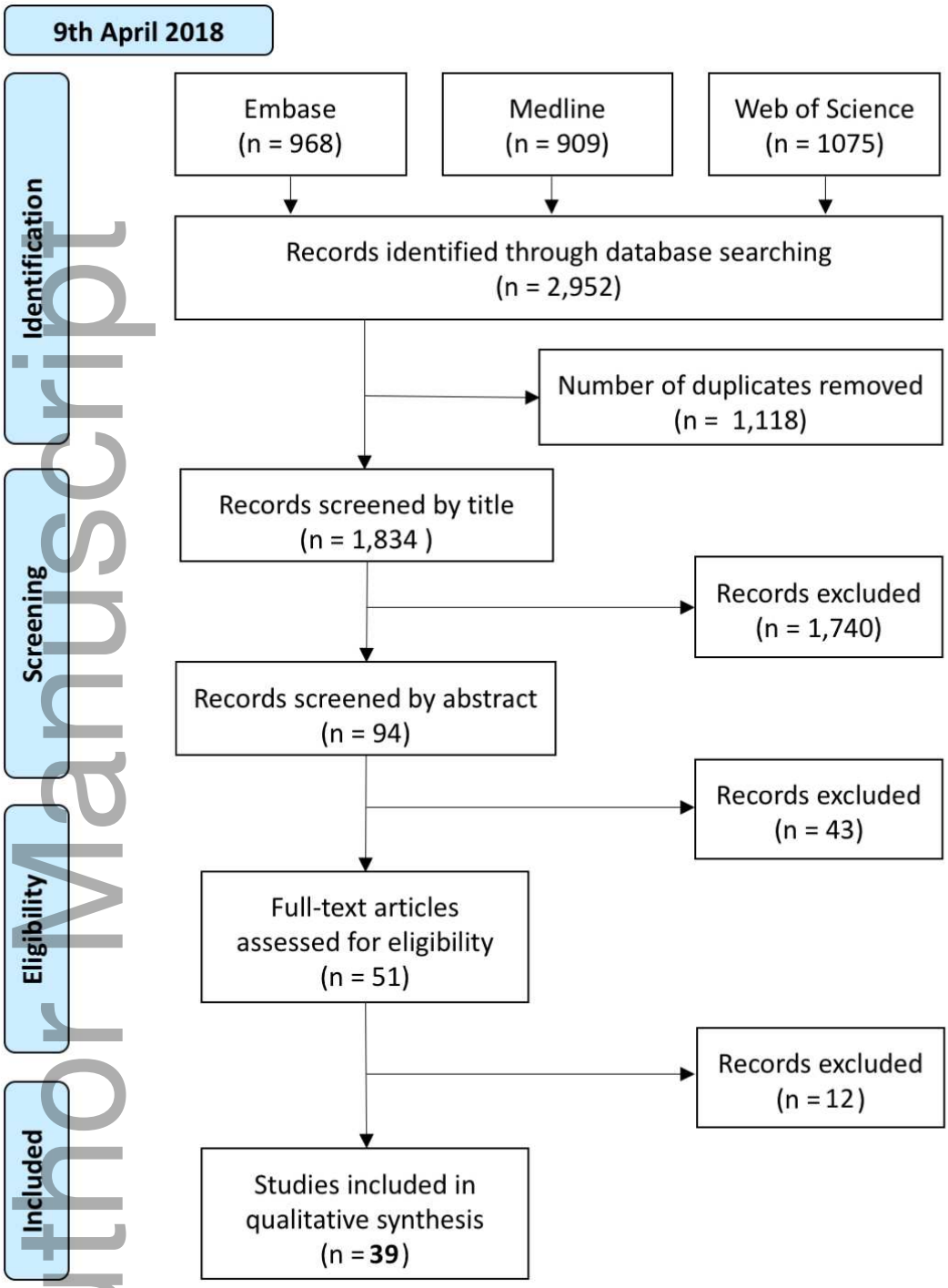


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