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# Mechanism of Apoptosis Induction in Oral Cancer Cells by Uncaria-derived Flavonoids

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***ABSTRACT***

***Introduction****: Oral cancer is cancer that occurs in the oral cavity and oropharynx, ranking 16th as the most common cancer found with a number of new cases of 377,713 and a number of deaths of 177,757 cases in 2020. Several types of flavonoids contained in the genus Uncaria were found to have anticancer bioactivity, one of which is inducing apoptosis in oral cancer cells.* ***Aim****: This study aims to review the potential and mechanisms involved of flavonoids in inducing apoptosis in oral cancer cells. The literature review method was used in this study by conducting a systematic literature search on PubMed, ScienceDirect, and Google Scholar until October 2023.* ***Results****: A total of 13 studies were included in this study and our findings conclude that flavonoids, especially quercetin, kaempferol, rutin, umbelliferon, and epigallocatechin gallate (EGCG), have the potential to be used as anticancer agents, one of which has the ability to induce apoptosis, both through extrinsic and intrinsic pathway, in several oral cancer cell lines, including SCC-4, SCC-9, SCC-25, SAS, KB/VCR, Tca-8113, YD10B, YD38, MC-3, KB, and HSC-3, through the mechanism of inhibiting anti-apoptotic expression, activating pro-apoptotic protein expression, generating ROS, and regulating PI3K/AKT, MAPK, and JAK/STAT signaling pathways.* ***Conclusion****: We conclude that Uncaria-derived flavonoids hav the potential to induce apoptosis, both extrinsic and intrinsic, in oral cancer cells.*

***KEYWORDS****: Apoptosis, flavonoids, oral cancer, uncaria*

## INTRODUCTION

Oral cancer encompasses a diverse array of types of cancer that can occur in different sites of the oral cavity and oropharynx, including the lips, gingiva, floor of the mouth, base of the tongue, palate, buccal mucosa, tonsils and throat walls (Y.-C. Huang et al., 2023). Oral cancer ranks 16th as the most common cancer found with a number of new cases of 377,713 and a number of deaths of 177,757 cases in 2020, with an incidence rate of 4.1 and a mortality rate of 1.9 per 100,000 (World Health Organization, 2020). There are various risk factors for oral cancer, including smoking or tobacco, consumption of alcohol, human papillovirus (HPV) infection, radiation exposure, genetics, inflammation, Fanconi anemia, oral microbiome, xeroderma pigmentosum, premalignant lesions, field cancerization, discoid lupus erythematosus,

scleroderma, diabetes and an unhealthy lifestyle (Feller & Lemmer, 2012; Irani, 2020; Miloro et al., 2011; Petti, 2009; Scully & Bagan, 2009).

The current main therapeutic modalities for oral cancer include surgical intervention, radiation therapy, and chemotherapy, which can be administered either alone or in combination with one another (Nandini et al., 2020). Nevertheless, current contemporary cancer therapy has various adverse side effects that can significantly diminish the patient's quality of life. Side effects induced by radiotherapy and chemotherapy include oral mucositis, hyposalivation, loss of taste, trismus, osteoradionecrosis, xerostomia, ulceration, secondary infection, bleeding due to oral mucositis, secondary malignancy, nausea, vomiting, anorexia, diarrhea, gastrointestinal toxicity, mobility abnormalities, cognitive impairment, nephrotoxicity, hepatotoxicity, neutotoxicity, cardiotoxicity, and ototoxicity (Barazzuol et al., 2020; Fernando & Jones, 2015; Kawashita et al., 2020; Singh & Bagewadi, 2022; van den Boogaard et al., 2022). Thus, there is a need for novelty in cancer treatment in order to reduce the incidence of bad side effects induced by current cancer therapy. Herbal medicines are believed to have cost effectiveness, high bioavailability, safety, and very minimal risks and side effects for patients (Abotaleb et al., 2018; Mosaddad et al., 2021) and possess the capacity to serve as alternative pharmaceuticals in the management of oral cancer.

Flavonoids are polyphenolic phytochemical compounds which are highly prevalent secondary metabolites in the genus Uncaria which possess significant promise for treating a wide range of diseases (Liang et al., 2020; Ridho, 2023). The potential of flavonoids as anticancer, antiproliferative, antidiabetic, anti-inflammatory, antihypertensive, anticholin-esterase, antibacterial, antiviral and antioxidant has been reported to be used as a pharmacological agent (Karak, 2019; Rahaman & Mondal, 2020). Several studies reported that flavonoids have chemopreventive effects by impeding the proliferation of cancer cells with the mechanism of estrogenic or antiestrogenic activity, preventing oxidation, anti-inflammation, modulation of the host immune system, anti-proliferation and promotion of apoptosis, inducing cell cycle arrest, inducing detoxification enzymes, and alteration of cellular signaling (Y.-C. Huang et al., 2023; Khan et al., 2021).

The development of herbal medicine using phytochemicals has grown rapidly at this time, one of which is used in alternative therapy for oral cancer. One of the therapeutic targets used to prevent the development of oral cancer cells is through the induction of apoptosis. Unfortunately, there is still very little research on the flavonoids contained in the Uncaria genus. Thus, the objective of the present study

is to review the potential of flavonoids derived from Uncaria and the mechanisms involved in inducing apoptosis in oral cancer cells. So, it can describe the potential and mechanisms involved, as well as provide evidence for use in future research.

## METHODS

The literature review method was used in this research. We conducted a systematic literature search on PubMed, ScienceDirect, and Google Scholar until October 2023 using the following combination of keywords: "flavonoids" AND "uncaria" AND ("oral cancer" OR "oral cancer cell" OR "oral squamous cell carcinoma") AND "apoptosis".

In searching and selecting studies, we applied inclusion criteria in the form of articles in Indonesian and English, articles published until October 2023, peer-reviewed research articles and not literature reviews, articles that can be accessed in full, otherwise articles were excluded. In the article selection process, we used the Mendeley reference manager to remove duplicates and reviewed the titles and abstracts to see the relevance of the articles to our research. The results of the included articles are then subjected to a more in-depth study in the discussion to produce a comprehensive summary.

In the search results, 17,700 articles were obtained from PubMed, ScienceDirect, and Google Scholar. After removing duplicates using Mendeley reference manager, applying the predetermined inclusion criteria, reviewing the titles and abstracts of the remaining articles to see the relevance of the articles, as well as assessing the eligibility of the articles, a total of 13 articles were finally included for review in this study.

## RESULTS AND DISCUSSION

### Uncaria-derived Flavonoids

Flavonoids are polyphenolic phytochemical compounds commonly found in several parts of plants, such as leaves, fruit, roots and stems (Karak, 2019). In plants, flavonoids protect plants from environmental influences as plant protectors from exposure to ultraviolet light, detoxification agents, antimicrobial defense compounds, and as functional roles in plant heat acclimatization and freezing tolerance (Panche et al., 2016). Flavonoids are categorized into several subgroups according to the carbon of the C ring to which the B ring is attached as well as the degree of unsaturation and oxidation of the C ring, namely flavanones, flavanonols, flavans, chalchones, anthocyanidins, isoflavonoids, and flavanones and flavanols which are included in anthoxanthins (Figure 1) (Ullah et al., 2020).

In plants of the genus Uncaria, 40 types of flavonoids have been identified (Q. Zhang et al., 2015) and much evidence reported that they have bioactivity, one of which is anticancer. Some of the main internal factors contributing to cancer are oxidative stress, genetic mutations, hypoxia, and apoptotic dysfunction, while external factors are

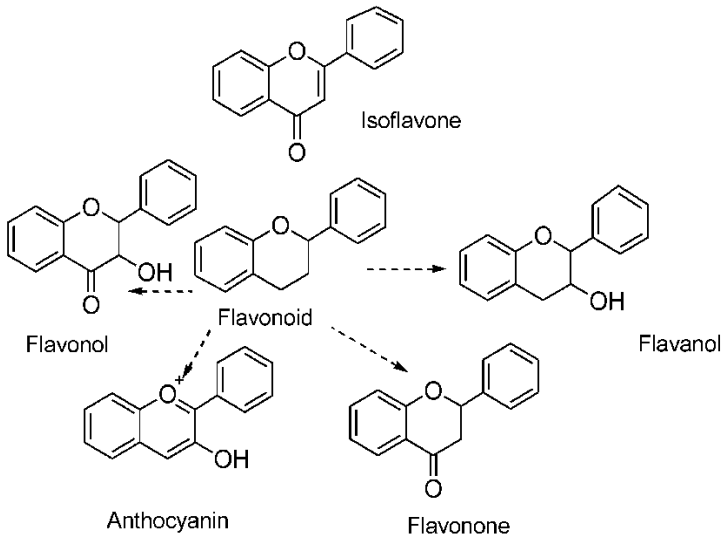


Figure 1. Structure and types of flavonoids (Ullah et al., 2020).

associated with heightened exposure to stress, radiation, pollution, UV light, and several other risk factors (Blackadar, 2016). Meanwhile, the characteristics that are characteristic of cancer include changes in metabolism, disruption of the cell cycle, mutations, immune response resistance, chronic inflammation, metastasis, and induction of angiogenesis (Neagu et al., 2019). The anticancer effects resulting from flavonoids are diverse, such as modulating ROS-scavenging enzymes, stopping the cell cycle, induction of apoptosis, autophagy, as well as reducing the proliferation and invasion activity of cancer cells (Kopustinskiene et al., 2020; Raffa et al., 2017).

In general, several types of flavonoids that have anticancer activity include flavonols, including quercetin, myricetin, kaempferol, isorhamnetin, and ampelopsin; types of flavanols such as epigallocatechin gallate (EGCG); types of flavanones such as hesperidin; types of flavones such as baicalein, acacetin, genkwanin, oroxylin A, pectolinarigenin, and galangin; types of isoflavones such as genistein and lupiwighteone; and types of anthocyanins, including delphinidin, cyanidin, and pelargonidin (Zughaibi et al., 2021). In the genus Uncaria, the most abundant types of flavonoid compounds are quercetin and kaempferol (Q. Zhang et al., 2015) and they are the types of flavonols that are often found in the Uncaria genus (Ridho, 2020). However, apart from quercetin and kaempferol, which are two compounds that are widely found, there are several types of flavonoids in the genus Uncaria which have the potential to induce apoptosis in oral cancer cells, as listed in the Table 1.

**Apoptosis in Oral Cancer**

Apoptosis is a programmed process of cell death mediated by caspases which cleave target proteins (Pfeffer & Singh, 2018). Apoptosis involves two distinct pathways,

Table 1. Flavonoids found in species of the genus Uncaria.

| Compound | Uncaria Species | Reference |
|---|---|---|
| Quercetin | *U. sinensis, U. cordata, U. gambir, U. tomentosa, U. rhynchophylla* | (Abdullah et al., 2016; Alfani et al., 2023; Kolodziejczyk-Czepas et al., 2021; Liang et al., 2020; Saad et al., 2020; Q. Zhang et al., 2015) |
| Kaempferol | *U. sinensis, U. cordata, U. tomentosa* | (Abdullah et al., 2016; Kolodziejczyk-Czepas et al., 2021; Q. Zhang et al., 2015) |
| Rutin | *U. hirsuta, U. elliptica, U. rhynchophylla, U. guianensis* | (Hernandes et al., 2020; Q. Zhang et al., 2015) |
| Umbelliferone | *U. hirsuta, U. sessilifructus, U. macrophylla* | (Q. Zhang et al., 2015; Z. Zhang et al., 2023) |
| EGCG | *U. gambir* | (Alioes et al., 2019; Manik et al., 2023) |

namely the extrinsic pathway which relies on transmembrane receptors for signaling, and the intrinsic pathway which takes place within the mitochondria (Dwivedi et al., 2020). The downregulation of apoptosis is primarily due to two factors. Firstly, it is produced by the occurrence of somatic and non-somatic mutations, as well as the decreased or lost expression of pro-apoptotic proteins. Secondly, it is a consequence of the excessive expression of anti-apoptotic proteins (Jain et al., 2013).

In oral cancer cells, BCL-2 associated X (BAX) protein expression has been shown to be decreased and B-cell lymphoma-extra large (BCL-xL) expression has been reported to be overexpressed and can result in resistance to several chemotherapy agents (Misra et al., 2016). Caspase-3 in oral cancer cells is downregulated, thereby inhibiting protein cleavage that activates apoptosis (Hague et al., 2004). The study carried out by Coutinho-Camillo et al. stated that all anti-apoptotic proteins, consisting of B-cell lymphoma (BCL)-2, BCL-x, BCL-xL, BCL-2-related protein A1, BCL-2 associated athanogene (BAG)-1, survivin, and pro-apoptotic proteins, including BAX, BCL-2 antagonist/killer (BAK), BCL-2 associated death promoter (BAD), BH3 interacting-domain death agonist (BID), BCL-2 interacting mediator of cell death (BIM), BIM-Long, p53 upregulated modulator of apoptosis (PUMA), apoptotic protease activating factor (APAF)-1, caspase-2, -3, -6, -7, -8, -9, -10, second mitochondria-derived activator of caspase (smac)/direct inhibitor of apoptosis-binding protein with low pl (DIABLO), as well as cytochrome C (CytC) are all expressed in oral squamous cell carcinoma (Coutinho-Camillo et al., 2017).

Furthermore, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) axis is recognized to have a role in the oral carcinogenesis progression by inhibiting apoptosis through the promotion of nuclear factor kappa B (NF-κB) (Sinevici & O'sullivan, 2016). The PI3K/AKT is a cellular signaling system that promotes cell survival by

inhibiting apoptosis in various types of cells (Lee et al., 2016). Targeting the suppression of the PI3K/AKT pathway is important due to its potential as a combination therapy that effectively inhibits all aberrant downstream signals that oral cancer cells need to survive and grow (Aggarwal et al., 2019). Therefore, therapies targeting the PI3K/AKT pathway inhibition are considered to be quite promising.

The mitogen-activated protein kinase (MAPK) pathway comprises extracellular signal-regulated kinase (ERK)1/2, c-Jun N-terminal kinase (JNK)1/2/3, and p38 MAPK (Leelahavanichkul et al., 2014). In the cell apoptosis mechanism, the MAPK signaling pathway will suppress, directly, the activation of caspase-3 which is an effector of apoptosis, or indirectly by activating BCL-2 family proteins. In addition, this signaling pathway will inhibit mitochondrial CytC release and indirectly reduce downstream caspase-3 activity. Thus, this will prevent the apoptosis mechanism in cancer cells (Peng et al., 2017). Suppressing of the MAPK signaling pathway has the capacity to inhibit the proliferation of cancer cells and trigger apoptosis in oral cancer.

**Mechanism of Uncaria-derived Flavonoids in Apoptosis**

After conducting a literature search, a total of 13 articles were included, consisting of 8 articles discussing quercetin, 2 articles discussing kaempferol, 1 article discussing rutin, 1 articles discussing umbelliferone, and 2 articles discussing EGCG. All articles are *in vitro* studies using oral cancer cell lines (Table 2).

Quercetin can induce apoptosis in oral cancer cells by reducing the expression of BCL-2 (C.-F. Huang et al., 2022; Ma et al., 2018; Yuan et al., 2015) and increasing BAX expression (C.-F. Huang et al., 2022), resulting in a decrease in the BCL-2/BAX ratio, which is an indicator in the regulation of CytC release from mitochondria (Sharifi et al., 2014), so that a decrease in the BCL-2/BAX ratio due to the effects of quercetin will induce apoptosis in oral cancer (He et al., 2022). Additionally, the pro-apoptotic proteins expression was also induced by quercetin, such as BAK (C.-F. Huang et al., 2022; Yuan et al., 2015) and BID, whereas the expression of anti-apoptotic BCL-xL was inhibited (Ma et al., 2018), thus when these pro-apoptotic proteins are activated and anti-apoptotic proteins are decreased, it will activate apoptosis (Dwivedi et al., 2020).

Through the extrinsic pathway, quercetin also possesses the capability to enhance the expression of several proteins, including FAS, FASL, and FADD (Ma et al., 2018). Subsequently, FAS will attach to FAS-associated death domain (FADD) and create a death-inducing signaling complex (DISC) (Sari, 2018), which will initiate caspase-8 activation, which is also induced by quercetin. The interaction between FAS-FADD will also influence the increase in stress received by the endoplasmic reticulum (ER) in cancer cells.

Table 2. Mechanism of action of Uncaria-derived flavonoids in inducing apoptosis in oral cancer cells from all included articles.

| Compound | Oral cancer cell | Mechanism of action | Reference |
|---|---|---|---|
| Quercetin | SCC-9 | Activates caspase-3 and induces S-phase arrest | (Haghiac & Walle, 2005) |
| Quercetin | SCC-25 | Increases the proportion of apoptotic cells, drastically reduces antiapoptotic BCL-2, increases BAX, and increases cleaved caspase-3 and PARP expression | (Chen et al., 2013) |
| Quercetin | SAS | Regulates PI3K, ERK1/2, p-ERK1/2, JNK1/2, p38, and p-p38 protein expression | (Lai et al., 2013) |
| Quercetin | KB/VCR | Decreases BCL-2 expression and increases BAX and caspase-3 expression | (Yuan et al., 2015) |
| Quercetin | SAS | Increases the expression of FAS, FASL, FADD, caspase-8, BID, and CytC and decreases the expression of BCL-2 and BCL-xL | (Ma et al., 2018) |
| Quercetin | Tca-8113 | Downregulates the PI3K/AKT signaling pathway | (Lanfei et al., 2021) |
| Quercetin | SAS | Increases the expression of CytC, BAX, BAK, caspase-3, caspase-7, p-ERK1/2, p-JNK1/2, and PARP and decreases the expression of BCL-2 | (C.-F. Huang et al., 2022) |
| Quercetin | YD10B, YD38 | Increases the level of p-p38 | (Son & Kim, 2023) |
| Kaempferol | SCC-4 | Inhibits ERK1/2 phosphorylation | (Lin et al., 2013) |
| Kaempferol | MC-3 | Decreases ERK and increases p-JNK and p-p38 | (Jeon et al., 2023) |
| Rutin | SCC-4 | Regulates PI3K/AKT, MAPK, and JAK/STAT signaling pathways | (Predut et al., 2022) |
| Umbelliferone | KB | Generates ROS, interferes with ΔΨM, and releases CytC | (Vijayalakshmi & Sindhu, 2017a) |
| EGCG | SCC-4 | Activates BAD, BAK, FAS, IGF1R, WNT11, and ZEB1 gene expression, and inhibits caspase-8, MYC, and TP53 | (Irimie et al., 2015) |
| EGCG | HSC-3 | Increases caspase-3 and -7 activity | (Yoshimura et al., 2019) |

Increasing ER stress will increase calcium intake ($Ca^{2+}$), GRP-78, and ATF-6αβ (Ma et al., 2018). During the caspase cascade, the activation of caspase-3 and -8 will have an impact on the proteolytic cleavage of caspase-7 or -3, where caspase-3 serves as the primary executor in the mechanism of apoptosis (Veeravarmal et al., 2016), thereby promoting apoptosis. In this process, quercetin increases the activation of caspase-8, caspase-7, and caspase-3, thus inducing apoptosis in oral cancer cells.

Furthermore, caspase-8 activation, which is an initiator caspase, by quercetin will have an

impact on cleaving BID which will lead to the insertion of BAX into the mitochondrial membrane and the subsequent release of CytC which is also induced by quercetin (C.-F. Huang et al., 2022; Ma et al., 2018), smac/DIABLO, AIF, and Htr2 (Sari, 2018). Induction by BID, ATF-6 αβ, and poly(adenosine diphosphate-ribose) polymerase (PARP) is able to suppress systems that exist in cell mitochondrial organelles. Mitochondria as an ATP reservoir are crucial for the survival of cancer cells. The level of mitochondrial activity can be viewed through the mitochondrial membrane potential ($\Delta\psi_{M)}$ value parameter. The value reflects the membrane potential of mitochondria resulting from the Krebs cycle process, where the Krebs cycle plays an important role in the body's ATP production. Suppressing the $\Delta\psi_M$ value will be able to reduce the function of mitochondria as a reservoir for ATP producers, which will result in a decrease in ATP production. A cell where there is an imbalance between ATP production and the level of energy consumption will cause the cell apoptosis process to be faster.

In addition to triggering caspase-8 activation, prior studies have demonstrated that the bioactive compound quercetin can also activate caspase-3, which plays a pivotal role in orchestrating apoptosis. Caspase-3 has been associated with tumorigenesis and prognosis in OSCC. Aberrant expression of caspase-3 has been identified in multiple cancer types, with instances of both reduced and elevated expressions reported. The mitochondrial apoptosis pathway involves the Bcl-2 family, Bax proteins, and Bcl-2 expression. The balance between Bcl-2 and Bax proteins influences cell susceptibility to apoptotic cell death induction. Upstream apoptosis modulation affects PARP activity, leading to caspase 3 activation, which subsequently triggers downstream apoptosis. In SCC-25 cells, quercetin prompted apoptosis by inducing a persistent reduction in anti-apoptotic Bcl-2 levels and an elevation in proapoptotic Bax levels, leading to an increased Bax/Bcl-2 ratio. These findings underscore the significance of the Bax/Bcl-2 ratio in regulating the fate of oral cancer cells. Overall, quercetin predominantly impacts SCC-25 oral cancer cells by causing cell cycle arrest and triggering mitochondria-mediated apoptosis at both cellular and molecular levels (Chen et al., 2013; Haghiac & Walle, 2005).

In addition, PARP undergoes cleavage during the apoptosis process by caspase-3 and causes DNA damage and induction of apoptosis. The apoptotic process which is influenced by DNA strand damage or DNA synthesis will trigger the termination of the life cycle of specific cancer cells in the S or G1 phase. Cancer cells that do not develop in this phase will be exacerbated by severe ATP hypoxia because suppression of mitochondrial function will trigger cell apoptosis. PARP as one of the factors that triggers stress in mitochondria is able to trigger an increase in the production of strong oxidants such as ROS

in cells that originate from mitochondria. A significant increase in ROS levels will trigger accelerated cell apoptosis (Zhao et al., 2018). In this mechanism, PARP is induced by quercetin (C.-F. Huang et al., 2022), and both, PARP and caspase-3 activation, are indicators of apoptosis in oral cancer (Kim et al., 2015).

Likewise, quercetin has the ability to suppress the expression of PI3K/AKT in oral cancer cells (Lai et al., 2013; Lanfei et al., 2021). In addition to quercetin, other flavonoid compounds like rutin possess comparable capabilities in inducing apoptosis in OSCC cells through modulation of the PI3K/AKT signaling pathway (Predut et al., 2022). By suppressing the PI3K/AKT signaling pathway, it leads to activate p38 and JNK which results in activation of apoptosis (Fragoso & Barata, 2015; R. Zhang et al., 2018). The MAPK pathway, consisting of p38, ERK1/2, and JNK1/2, is also affected by quercetin (Lai et al., 2013) as well as kaempferol (Jeon et al., 2023; Lin et al., 2013) through the induction of p38, p-p38, JNK1/2 and p-JNK1/2, as well as inhibiting the expression of ERK1/2 and p-ERK1/2 in oral cancer cells. Induction of p38 and JNK will release CytC and result in caspase activation (Yue & López, 2020), so that the MAPK signaling pathway regulated by quercetin will result in the induction of apoptotic activity in oral cancer cells.

Apart from playing a role in inhibiting cellular genetic processes, the active compounds quercetin and kaempferol are able to block the P-gp protein on the cancer cells surface. The P-gp protein channel in cancer cells is responsible for maintaining the survival of cancer cells via the process of pumping chemotherapy drugs that successfully enter the cells. As a result, drugs that enter the cells will not have time to have a pharmacotherapeutic effect and can cause drug resistance in cancer cells. Through the barriers played by quercetin and kaempferol, they will inhibit the re-pumping process of drugs that have successfully entered the cells so that the chemotherapy drug compounds that have diffused into the cells will provide a pharmacotherapeutic effect by increasing the sensitivity of cancer cells to chemotherapy drugs and have an impact on acceleration of the apoptosis process (Yuan et al., 2015).

In addition to quercetin, the Uncaria plant contains other bioactive compounds, including EGCG. The induction of apoptosis occurs through the upregulation of Fas/CD95 expression. In conjunction with the increased expression of Fas/CD95, EGCG inhibits STAT3 phosphorylation and its translocation into the nucleus, resulting in the downregulation of STAT3 target gene products such as Bcl-2, Mcl-1, VEGF, and cyclin D1. Therefore, EGCG can trigger apoptosis by influencing both mitochondria-mediated and death receptor-mediated pathways. Caspases are a family of intracellular cysteine proteases crucial for initiating and executing apoptosis. The pathways mediated by mitochondria and death receptors converge at the level of caspase-3

activation, with caspase-3 and -7 acting as effectors of apoptotic cells. Our evaluation of EGCG's impact on caspase-3 and -7 activities revealed that the proapoptotic effect of EGCG in HSC-3 cells was mediated through the activation of these caspases (Yoshimura et al., 2019). Caspase-3, the primary executor of apoptotic cell death, inhibits the generation of ROS and is essential for effective cell elimination. Conversely, caspase-7 does not affect sensitivity to intrinsic apoptosis but contributes to ROS generation and cell detachment. Overall, caspase-3 and caspase-7 perform distinct roles in the apoptotic mechanism of OSCC. Caspase-3 is crucial for efficient cell elimination by inhibiting ROS production, while caspase-7 contributes to ROS production and cell detachment (Braicu et al., 2015; Yoshimura et al., 2019).

The bioactive compound EGCG exhibits a remarkable capacity to inhibit cell proliferation through apoptosis in a dosage-dependent manner. This effect has been validated across diverse cell lines, where EGCG not only promotes the expression of BAD, BAK, FAS, IGF1R, WNT11, and ZEB1 genes but also suppresses caspase-8, MYC, and TP53. TP53, a critical regulator in tumor cell dynamics, governs essential processes such as cell cycle control, cellular senescence, apoptosis induction, and autophagy modulation. Intriguingly, EGCG treatment leads to the downregulation of TP53, a phenomenon linked to increased sensitivity to chemotherapy, highlighting its potential in cancer treatment. A notable discovery has surfaced regarding the suppression of CASP8, a pivotal upstream controller engaged in death receptor-triggered apoptosis, which also plays a role in mitochondria-mediated apoptosis through the cleavage of proapoptotic factors. Simultaneously, EGCG activates CASP8 and enhances the functionality of the FAS/CD95 pathway, as demonstrated in our investigation of SSC-4 cells. The FAS gene plays a crucial role in the apoptotic machinery of oral cancer cells, with in vivo studies showing apoptosis upon local FAS activation in epithelial cells. Remarkably, the MYC gene has been implicated in orchestrating apoptosis in OSCC, potentially through upregulating FAS expression due to its dual role in inducing apoptosis and proliferation in normal cells (Braicu et al., 2015).

Additionally, BAD and BAK, which are proapoptotic components of the BCL-2 family, control the intrinsic pathway of apoptosis. BAK, which is essential for intrinsic apoptosis, is specifically activated by EGCG in SSC-4 cells. OSCC displays reduced BCL-2 expression compared to oral epithelium. Overexpression of ZEB1, which is known to inhibit cell proliferation and induce apoptosis, presents promising implications for novel therapeutic strategies, particularly in multitargeted therapy contexts. Furthermore, as tumors advance, the expression of IGF1R rises, while reducing IGF1R levels induces apoptosis, although it can also activate alternative antiapoptotic pathways and

contribute to cell proliferation and differentiation. IGF1R overexpression, significantly associated with OSCC carcinogenesis, may confer resistance to therapy. WNT11, which is overexpressed in OSCC, represents a potential therapeutic target due to its involvement in activating tumorigenesis through epigenetic alterations in WNT-pathway genes (Yoshimura et al., 2019).

In our literature investigation, the latest compound under study was umbelliferone, a flavonoid derivative. Umbelliferone demonstrates the ability to stimulate the generation of ROS, disrupt $\Delta\psi_M$, and initiate the release CytC. While ROS are typically perceived as harmful agents capable of causing various detrimental effects such as cellular dysfunction, death, or malignant transformation, research indicates that ROS also serve beneficial roles in multiple signaling pathways that govern development and uphold cellular equilibrium. Umbelliferone effectively enhanced ROS production in both cell types, leading to apoptosis. Intracellular ROS play a crucial role in promoting apoptosis by influencing DNA fragmentation and nuclear damage. Consequently, umbelliferone emerges as a promising anticancer agent that not only boosts peroxide production in normal cells but also counteracts the detrimental effects induced by cancer cells. This highlights umbelliferone's potential as an anticancer therapeutic for oral cancer, as it protects cells by enhancing their oxidative capacity, potentially through its anti-proliferative properties. Elevated ROS levels are particularly triggered when cells confront chemical or environmental stressors, which could be a pivotal factor leading to cell cycle arrest or apoptosis (Vijayalakshmi & Sindhu, 2017).

Mitochondria have a vital function in integrating and conveying signals of cell death to the caspase cascade, acting as a central regulator for key events in the apoptotic pathway. These events include disturbances in electron transport, ΔΨM disruption, and the release of caspases activators. Changes in ΔΨM are indicative of early apoptosis stages. Cancer cells typically display lower membrane potentials at rest compared to normal proliferating cells. Treatment of KB cells with umbelliferone led to notable morphological changes associated with apoptosis, such as apoptotic body formation and chromatin condensation. Previous studies have also documented umbelliferone-induced DNA damage in HepG2 cells, likely attributed to increased intracellular umbelliferone uptake resulting in elevated levels of ROS and subsequent ΔΨM disruption, ultimately leading to apoptotic morphological alterations. Our data support the notion that umbelliferone triggers apoptosis in KB cells through a pathway reliant on mitochondria. Experiments utilizing cell-free systems have shown that CytC release is a crucial event for activating caspases and endonucleases. Cytosolic CytC activates caspases, including caspase-3, thereby initiating apoptosis. Our study

provides evidence suggesting that umbelliferone-induced apoptosis in KB cells is mediated by ΔΨM disruption, enhanced CytC translocation to the cytosol, caspase-3 activation, and PARP degradation (Li et al., 1997).

Moreover, ROS play a crucial role in apoptosis by regulating particular enzymes associated with the cellular death process. These findings highlight the significant impact of intracellular oxidative byproducts on the control of apoptosis. Various stimuli, including anticancer agents, have been shown to stimulate ROS production within cells, initiating apoptosis mediated by ΔΨM disruption. The suppression of growth and the production of ROS caused by umbelliferone in KB cells suggest that ROS buildup probably initiates apoptotic cell demise via the mitochondrial pathway. The hallmark of oxidative stress is DNA damage by reactive oxygen byproducts. Research suggests that umbelliferone may function as an antimutagen by modulating DNA replication and repair processes following mutagen-induced DNA damage (Gedik et al., 1992; Tice et al., 2000; Yamashita & Kawanishi, 2000).

Based on the findings of a review of all articles included in this study, we conclude that the flavonoids contained in Uncaria, especially quercetin and kaempferol, have potential as agents in oral cancer therapy as proven by several evidences of the mechanisms and targets of quercetin and kaempferol in inducing apoptosis in several oral cancer cell lines. However, this review requires future research to analyze the flavonoid content derived from Uncaria other than quercetin and kaempferol to evaluate its anticancer bioactivity. Apart from that, research on the content of other secondary metabolites also needs to be carried out, as well as anticancer bioactivity besides induction of apoptosis, so that more comprehensive results will be obtained..

## CONCLUSION

We conclude that quercetin and kaempferol, a type of flavonoid derived from Uncaria, have the potential in apoptosis induction in oral cancer cells, through both extrinsic and intrinsic pathway, with the mechanism of inhibiting anti-apoptotic expression, activating pro-apoptotic protein expression, and regulating PI3K/AKT and MAPK signaling pathways. The findings of the present study contribute to comprehensive understanding the role of Uncaria-derived flavonoids and the mechanism involved in inducing apoptosis in oral cancer cells. The further studies are warranted to explore the stability, bioavailability and targeted delivery of these compounds to oral cancer cells. Clinical trials are also needed to evaluate safety and efficacy of these compounds to oral cancer treatment. The lack of research on flavonoid compounds from the genus Uncaria may be a consideration for further exploration of other flavonoid compounds regarding their potential to induce apoptosis in oral cancer

cells.

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