Hydroxychavicol as a potential anticancer agent (Review)

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Abstract. *Piper betle* leaves are widely cultivated in Malaysia, India, Indonesia and Thailand. They have been used as a traditional medicine for centuries due to their medicinal properties, including antioxidant, antiproliferative, antibacterial, antifungal and anti-inflammatory properties, which are attributable to their high phenolic contents. Hydroxychavicol (HC), a primary constituent of *P. betle* leaves, is known to possess antiproliferative activity at micromolar doses on various cancer cell lines of different origins while leaving normal cells unharmed. The present review summarises the mechanisms of action of HC reported in the literature, reviews the scope of work done thus far and outlines the direction of future research on the potential of HC as an anticancer agent. PubMed, Scopus and Web of Science were searched using the keywords (hydroxychavicol OR 4-allylpyrocatechol OR 4-allylcatechol) AND (cancer OR carcinogenesis OR tumour OR carcinoma) to acquire research articles. *In vitro* studies reported several possible mechanisms for the chemopreventive effects of HC against cancer cell lines, including chronic myelogenous leukaemia (CML), prostate, glioma, breast and colorectal cancers, while *in vivo* studies encompassed investigations on Ehrlich ascites carcinoma cells in Swiss albino mice and a CML mouse model. These studies suggest that HC exerts its anticancer effect via the modulation of mitochondrial membrane potential and the c-Jun N-terminal kinase, mitogen-activated protein kinase and endoplasmic reticulum-unfolded protein responses pathways and the generation of reactive oxygen species. In summary, future research should focus on combinations of HC with other anticancer drugs and testing in animal models to evaluate its bioavailability, potency and tissue and dose selectivity.

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Contents

1. Introduction
2. Methods
3. Composition of HC
4. *In vitro* and *in vivo* studies
5. Anti-inflammatory activity of HC
6. Conclusions and prospects

1. Introduction

For over 1,000 years, medicinal plants have attracted considerable interest as valuable resources for the development of novel therapies targeting various receptors and signalling pathways (1). The World Health Organization has recognised the importance of developing the knowledge base for the active management of traditional and complementary medicine (TCM) through national policies. Thus, substantial funding has been provided for TCM research (2).

Traditional medicinal plants are an essential component of indigenous medical systems globally. In Malaysia, ~1,300 plant species are suggested to have medicinal properties. However, only ~100 have been investigated for their medicinal potential. Thoroughly studying the benefits of these plants may enable them to serve as practical alternative medicines (3). Alternative therapeutic approaches have been applied using highly effective natural compounds that possess pleiotropic properties, such as targeting signalling mediators, transcription factors, growth factors and kinases that modulate multiple signal transduction pathways (4). In this respect, phytochemical extracts such as flavonoids, carotenoids and phenolic compounds from medicinal plants have been extensively investigated for their anticancer activities because they are often less toxic to healthy cells. Some phytochemicals are less potent than synthetic drugs (5-7). Therefore, phytochemicals are often investigated in combination with other chemotherapeutic agents to increase the efficiency of cancer treatment while reducing the adverse effects on healthy cells (8). However, a cautious interpretation of the benefits of phytochemicals is essential. Some phytochemicals are toxins that may act as carcinogens or tumour promoters (9). For example, studies have shown that the leaves of betel [*Piper betle* (Piperaceae)] cause oral cancer. However, the increased risk of oral cancer is due to the consumption of betel quid, a concoction including other carcinogenic

components, while betel leaves themselves possess anticancer properties (10).

The medicinal properties of betel leaves have long been known (11,12). However, investigations into the mechanism of action and the bioactive components with therapeutic potential are ongoing (12,13). *P. betle* is a perennial vine creeper commonly found in the tropical rainforest. Its leaves have been used in native ceremonies as spices and traditional medicine in India, Sri Lanka, Bangladesh, Thailand, Malaysia and Indonesia (11,14-16).

Betel leaves are an excellent mouth freshener (11). They are also considered to be helpful treatments for various ailments, including boils and abscesses, conjunctivitis, constipation, headache, itches, gum swelling and rheumatism (17). Furthermore, betel leaves have been shown to possess other bioactivities, including antidiabetic, antiproliferative, antitumour, anti-inflammatory and antimutagenic activities (12,18-21). Due to the potential of *P. betle*, research is proceeding on its bioactive components, including hydroxychavicol, eugenol, chavibetol and chavivol, which may be important for halting cancer growth or killing cancer cells via their chemotherapeutic or chemopreventive properties (10,22). Among these, hydroxychavicol (HC), also known as 4-allyl-catechol is a major catecholic component of betel leaves that has been shown to have strong antimutagenic properties when compared with eugenol (12).

HC has been reported to possess inhibitory properties against prostate, colon, glioma and leukemic cancer cells while leaving healthy cells unharmed (23). Notably, studies combining HC with other drugs have shown this to be an advantageous approach, since HC showed good results when integrated with other chemotherapeutic agents as an adjuvant. For example, when combined with γ-tocotrienol (GTT), HC exhibited multiple molecular effects on glioma cancer cells, including the suppression of cell proliferation by the endoplasmic reticulum (ER) untranslated protein response pathway via activation of transcription factor 4 and DNA damage-inducible transcript 3 (DDIT3) protein (24). Due to the pleiotropic effects of HC on various cancer cell lines and animal models (25-31), the mechanisms of action of HC described in the literature were examined in the present review to summarise the current study scope. Thus, the present review may provide critical insights into the cancer-preventive potential of HC, such as its antioxidant, antiproliferative and anticancer effects. The study encompasses the fundamental chemistry and biochemical activity of HC, laboratory studies and the mechanism of action of HC *in vitro* and *in vivo*.

2. Methods

The present scoping review followed five steps: i) Definition of the research question; ii) identification of relevant studies; iii) selection of articles to include in the review; iv) charting the information; and v) summarising and reporting the results (32). This scoping review complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (33). Articles were identified in August 2022 using PubMed, Scopus and Web of Science using the search string (hydroxychavicol OR 4-allylpyrocatechol OR 4-allyl-catechol) AND (cancer OR carcinogenesis OR tumour OR

carcinoma). From these databases, 156 articles were retrieved, encompassing publications between 1986 and 2022. After the removal of 75 duplicates, 81 unique articles were identified and subjected to screening. Of these 81 articles, nine were excluded due to being inappropriate article types, such as chapters in books or review articles. Another 40 articles were removed due to being on unrelated topics, such as those not on HC, articles focused on *P. betle* only instead of HC, studies focused on other properties of HC (for example as an antibacterial or oral care agent) and studies on extraction methods. Following evaluation of the full text of the remaining 32 articles, 12 were rejected because they were not on cancer cells or cancerous animal models or were studies associated with HC derivatives. Finally, the present review included 20 articles for analysis, as shown in the PRISMA flow chart in Fig. 1.

The publications were organised and duplicate articles were identified using EndNote 20 (Clarivate). Article titles and abstracts were evaluated independently by two authors (AAR and NAM) prior to retrieval of the full text for further review based on the inclusion and exclusion criteria. Any differences in opinion between the two authors regarding article inclusion were discussed with the third author (SHSAK) to resolve the conflict. Two authors (AAR and NAM) extracted data encompassing information on authors, years, study design, experiment model type and notable findings.

3. Composition of HC

HC is the most abundant component of betel leaves (11), and can be extracted using various chemical solvents such as ethanol, methanol and chloroform, or by aqueous extraction. Methanol extraction has been shown to yield a higher concentration of HC (25.035 mg/g) than ethanol, ethyl acetate or n-hexane (34). The ethanol extract of betel leaves reportedly contains 66.6% HC, followed by 11.9% eugenol, indicating that HC is the primary constituent of these leaves (35,36). In another study comparing the extraction of HC with methanol, boiling water, hexane (10% ethyl acetate), chloroform, solvent-free microwave and hydrodistillation, it was found that boiling water extraction provided the highest yield of HC (98%) while hydrodistillation gave the lowest (2%) (37). In general, HC remains stable in the dried extract when stored at 5˚C in the dark, and its antioxidant activity is stable after 180 days of storage under suitable conditions (38). Furthermore, HC is soluble in non-polar solvents due to the presence of two hydroxyl (-OH) groups in its chemical structure (34) (Fig. 2). Table I summarises the properties of HC.

Structurally, HC has an aromatic core with 1,2-dihydroxyl substituents forming a catecholic structure with redox properties, enabling it to behave as an electron acceptor or hydrogen donor. These characteristics are due to radical structure resonance and oxyanion stabilisation, respectively. In cancerous cells, this redox feature may be crucial in reducing the levels of reactive oxygen species (ROS) and preventing the cells from becoming malignant (39). In addition, HC has been suggested to induce DNA damage via the chelation and reduction of iron or copper ions, or via the *ortho*-hydroxy phenoxy radical produced by oxidation of the catechol moiety of HC with $^3O_2$ (40). Notably, HC can act as an antioxidant for cells at different concentrations (39).
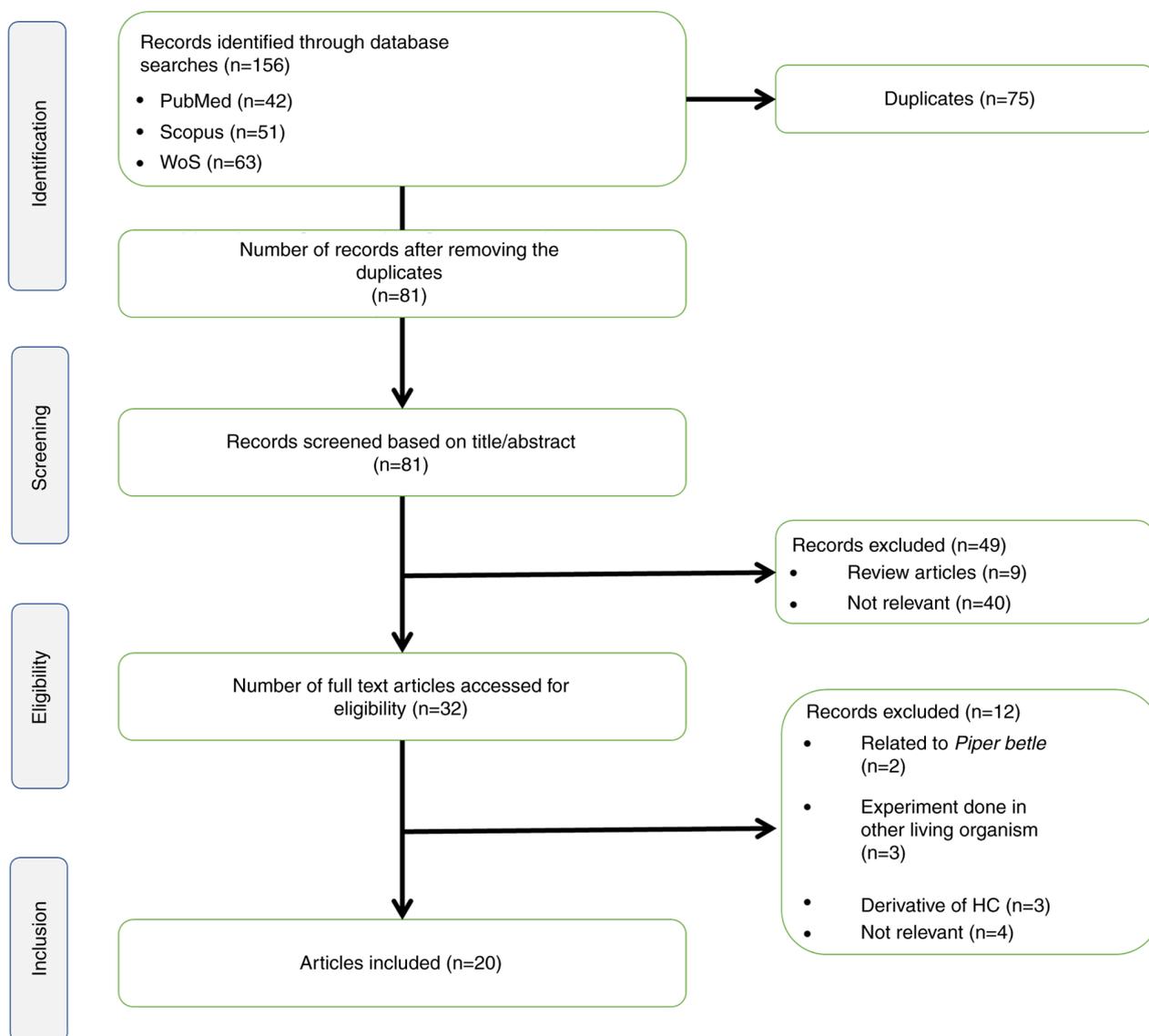
Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis flow diagram depicting the identification and selection of articles. WoS, Web of Science; HC, hydroxychavicol.
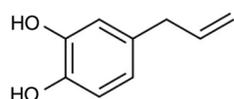

Figure 2. Chemical structure of hydroxychavicol drawn using ChemDraw.

4. *In vitro* and *in vivo* studies

Cancer occurs partly due to imbalanced ROS levels and an inefficient antioxidant defence system, which generate unstable free radicals and create an oxidative stress environment for cells (25,41). The detrimental effects of ROS on DNA, combined with an aberrant DNA repair system and certain signalling pathways, trigger mutations in cells and the development of cancer. During normal metabolism, ROS are produced in living organisms where they act as signalling molecules to activate cell proliferation, survival, apoptosis, differentiation, immune responses, motility and stress-responsive pathways (42,43).

Cancer cells circumvent the ROS regulation mechanisms by avoiding the usual thresholds for the induction of cell death by increasing their antioxidant levels aberrantly, thus enabling them to maintain high levels of ROS and optimise ROS-driven proliferation (44). However, the death of cancer cells can be induced by disrupting their redox homeostasis via the strong elevation of ROS levels or inhibition of the cells' antioxidant processes (Fig. 3). These disruptions can cause the extensive irreversible breakage of single- or double-stranded DNA, base modifications and DNA cross-links (45). HC potentially suppresses cell growth and proliferation in several cancers, including leukaemia, prostate, breast, pancreatic and brain cancers, via pleiotropic mechanisms, including antioxidant pathways. The effects of HC on various cancer models and the affected pathways are addressed in the following sections.

Chronic myelogenous leukaemia cancer (CML). Approximately 90% of cases of CML in patients are caused by the reciprocal translocation of chromosome 9 and chromosome 22 t(9;22)

Table I. Properties of hydroxychavicol.

Property	Hydroxychavicol
IUPAC Name	4-Prop-2-enylbenzene-1,2-diol
Molecular formula	$C_9H_{10}O_2$
Molecular weight	150.17 g/mol
Boiling point	316.77˚C
Melting point	166.14˚C

IUPAC, International Union of Pure and Applied Chemistry.

(q34;q11) that produces a chimeric Bcr-Abl gene (46). Patients with CML are generally treated with the first-generation tyrosine kinase inhibitor imatinib mesylate (IM). However, only 10% of the recipients showed positive responses, while others were resistant to IM treatment (47). Thus, efforts have been made to identify potential alternative and/or combined treatments for CML.

A study of imatinib-resistant CML cells (48) suggested that HC sensitised the cells to apoptosis via the induction of tumour-necrosis-factor-related-apoptosis-inducing ligand (TRAIL) mediated by ROS homeostasis. In the study, imatinib-resistant K562 cells were treated with 4 μM of HC alone or combined with 200 ng/ml TRAIL. Treatment with HC or TRAIL alone for 24 h induced only 5-10% cell death, while combined HC and TRAIL treatment for 24 h induced a significant cytotoxic effect with an increase in apoptosis of up to 81% (48). ROS played a primary role in the HC-mediated sensitisation of the cells to TRAIL, probably acting via the anti-apoptotic proteins X-linked inhibitor of apoptosis protein and FLICE-inhibitory protein. This was evident by the dose-dependent increase in ROS levels when the imatinib-resistant K562 cells were treated with HC and TRAIL together compared with the single HC treatment. However, ROS production was not affected by TRAIL (48).

Another study found that HC induced ROS in a time-dependent manner, resulting in ROS build-up and a state of high oxidative stress, which subsequently triggered phosphorylation of the c-Jun N-terminal kinase (JNK) pathway. The activation of JNK signalling by HC was suggested to induce the phosphorylation of endothelial nitric oxide synthase (eNOS), resulting in the generation of nitric oxide (NO) and, ultimately, cell death (25). However, HC alone did not increase extracellular signal-regulated kinase (ERK) levels or the activation of p38 in K562 cells, suggesting that the anticancer effect of HC is mediated by a mitochondrial ROS-dependent eNOS-mediated route (25). However, in another study, the activation of JNK with a combination of HC and buthionine sulfoximine (BSO) for 24 h stimulated the ERK pathway (49). Furthermore, the intracellular glutathione (GSH) level of the K562 cells was not affected by the HC treatment (25).

HC exhibited potent anti-CML effects in an *in vivo* nude mouse model with xenografts formed from leukaemia cell lines expressing wild-type and mutant Bcr-Abl. The results showed that the oral administration of 100 mg/kg HC twice daily for 5-7 days reduced subcutaneous tumour growth in the xenograft-bearing animals (25). Furthermore, HC was able to kill CML cell lines harbouring mutant T315I, one of the most common mutations causing imatinib resistance in patients, via the activation of JNK, leading to increased NO production via the phosphorylation of eNOS.

There is evidence that HC interacts synergistically with other treatments; for example, a study showed that 100 μM BSO combined with 10 μM HC potentiated CML cell death by disrupting the redox equilibrium of cells via the reduction of intracellular GSH and promotion of ROS generation. Furthermore, the combination of BSO and HC acted via the GSH-ROS-JNK-ERK-iNOS pathway and the partial activation of caspase-3 and poly (ADP-ribose) polymerase (PARP) proteins (49). Reactive nitrogen species produced by iNOS overexpression may also be involved in the induction of apoptosis in CML (49). This combined treatment might improve upon the efficacy of standard chemotherapeutics in halting the progression of CML cells.

In another study, a combination of 5 μM HC with 5 μM curcumin induced the accumulation of superoxide and H_2O_2, activated JNK and p38 in the mitogen-activated protein kinase (MAPK) cascade and induced the phosphorylation of mammalian target of rapamycin (mTOR) and its downstream mediators S6 kinase and eukaryotic translation initiation factor 4E binding protein 1, causing apoptosis in K562 cells (50). Although it was suggested that ER stress might induce apoptosis via activation of the mTOR signalling pathway, the underlying mechanism remained unclear. When used singly, these doses of HC and curcumin did not induce apoptotic changes in the CML cells. Also, treatment of the cells with apoptosis-mediating concentrations of HC alone inhibited mTOR signalling, while the combined treatment with HC and curcumin activated both mTOR and MAPK pathways; these effects were dependent on the generation of ROS and led to the apoptosis of CML cells (50).

Breast cancer. Breast cancer is the most frequently diagnosed cancer worldwide, with an estimated number of cases of over two million in females in 2020 (51). Breast cancers are classified into several molecular subtypes based on various characteristics, including their histology, metastatic potential, mutations, disease progression and response to therapy (52). Numerous chemotherapies inhibit the development of breast cancer by preventing DNA damage (53) or by blocking oestrogen activity, with examples including tamoxifen (54) raloxifene (55), aromatase inhibitors (56), polymerase inhibitors (57), human epidermal growth factor receptor 2 inhibitors and trastuzumab (58). Unfortunately, due to the severe side effects or chemoresistance that they induce, some of these treatments do not improve patients' morbidity or mortality (59).

A recent study indicated that HC acted as an antioxidant on Ehrlich ascites carcinoma (EAC) cells in an *in vivo* experiment using Swiss albino mice. The EAC-inoculated mice exhibited a high malondialdehyde level, indicating elevated ROS generation (60). The study showed that when administered orally for 21 days at doses of 200 and 400 mg/kg body weight, HC effectively reduced the volume of the EAC tumours in mice, indicating its anticancer potential. In addition, as the level of oxidative stress was significantly reduced, the lifespan of the tumour-bearing mice was increased (60).
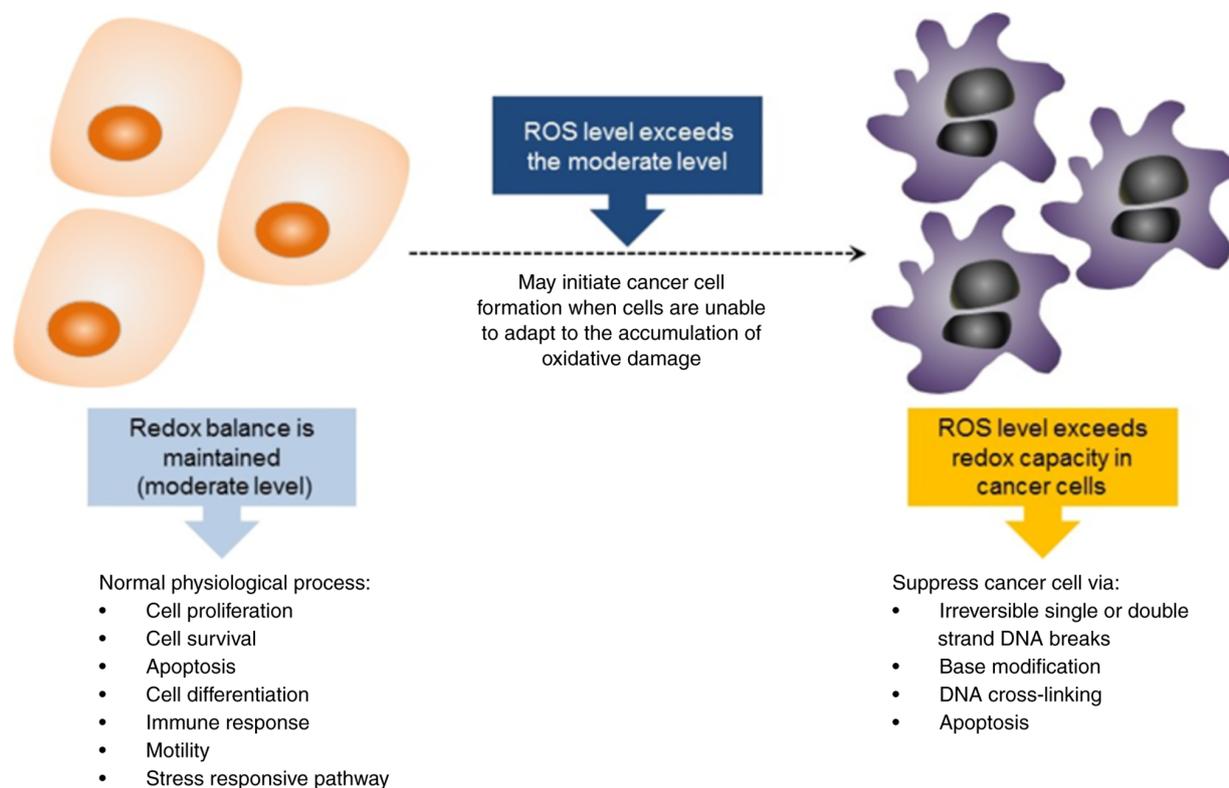
Figure 3. Redox balance of ROS in normal cells, the development of cancer cells and cancer suppression. ROS, reactive oxygen species.

Pancreatic cancer. Pancreatic cancer is the 14th most common cancer and the 7th most frequent cause of cancer-associated death worldwide (61). The standard treatment for advanced pancreatic cancer is gemcitabine (62). However, adjuvant therapy using a combination of oxaliplatin, irinotecan, leucovorin and fluorouracil, known as FOLFIRINOX, is also used due to its efficacy and ability to promote significantly longer survival than gemcitabine (63). Unfortunately, ~80% of cases of pancreatic cancer progress into metastatic cancers with the development of liver metastases within 2 years of the treatment regime, inclusive of adjuvant therapy (64). Thus, the search for an alternative treatment or adjuvant continues to be crucial.

The proliferation, migration and invasion of pancreatic cancer cells can be inhibited by HC treatment, as is evident by the inhibition of genes associated with epithelial-mesenchymal transition, i.e., goosecoid homeobox and snail family transcriptional repressor 2, and increased DNA damage as revealed by the comet assay (27). The HC concentration used for the migration assay and gene expression analysis was 25 μM for 24 or 48 h. However, higher HC doses were used for protein analysis, i.e., 50 and 100 μM for 48 h. The induction of apoptosis was confirmed by the activation of caspase-3, -8, and -9, the cell cycle proteins cyclin B1 and cell division control 2, cell division cycle 25C and apoptotic-associated proteins, including PARP, BH3 interacting domain death agonist, B-cell lymphoma 2 (Bcl2), Bcl2 associated X and survivin. The induction of apoptosis was suggested to be JNK-dependent. Furthermore, proteins associated with DNA damage, including Ser-139-phosphorylated H2A histone family member X and p53 binding protein 1, and genes DDIT3 and DNA

polymerase β were upregulated in MIA PaCa-2 and PANC-1 pancreatic ductal adenocarcinoma (PDAC) cells treated with HC. Notably, HC exhibited higher half-maximal inhibitory concentration (IC$_{50}$) values, indicating lower toxicity, in a panel of non-cancerous cells comprising L929 and NIH-3T3 mouse fibroblasts and human 293 cells compared with PDAC cells (27).

Prostate cancer (PCa). Aside from active surveillance, which is a viable method of management for localised PCa, the two primary curative forms of therapy are radical prostatectomy and radiotherapy (65). A review of prostate cancer therapies has shown that majority of patients receiving androgen-ablative or -deprivation therapy initially responded to treatment but became resistant over time and the cancer progressed (66).

In one study, the antiproliferative action of HC against a panel of prostate cancer cells comprising PC-3, C4-2, DU145 and 22Rv1 was shown to be dose- and time-dependent, with IC$_{50}$ values ranging from 30 to 320 μM (67). The HC treatment caused the PCa cells to accumulate in the G1 phase, signalling the onset of apoptosis (67), as evidenced by the upregulated levels of cleaved caspase-3 and cleaved PARP observed in PC-3 cells treated with HC for 18 h. HC was suggested to trigger cell death by generating high levels of ROS, which was further supported by a reduction in the mitochondrial transmembrane potential of PC-3 cells (67). Autophagic signalling was also postulated as an alternative pathway for the mechanism of action of HC in PCa cells since acidic vesicular organelles and the elevated expression of the autophagic markers light chain 3-IIb and beclin-1 were observed. Furthermore, the oral administration of 150 mg/kg reduced the tumour volume

of PCa xenografts in mice by ~72% (67). In addition, the HC dosage was well tolerated with no signs of toxicity, and the IC_{50} value of HC in RWPE normal prostate epithelial cells was 398 μM, which was much higher than that in the PCa cell lines (67).

Colorectal cancer (CRC). As of 2020, CRC ranked third and second among the most common cancers in men and women, respectively (51,68). Chemotherapeutic drugs for CRC include 5-fluorouracil and oxaliplatin. However, over time multidrug resistance may develop in some patients (69). Therefore, the identification of alternative compounds with anticancer potential that can overcome the multidrug resistance pathway is crucial.

Similar to findings in other types of cancer (67), HC induced cell cycle arrest at the G0/G1 and G2/M phases in TP53-resistant HT-29 colon cancer cells, causing cells to accumulate in the G0/G1 phase (70). The apoptotic cell death observed in HT-29 cells following treatment with 30 μg/ml HC was higher than that of HT-29 cells treated with 50 μmol/l 5-fluorouracil for 12, 18, 24 and 30 h (70). The HT-29 cells treated with HC exhibited increased activation of JNK and p38 MAPK following 12 h of treatment while the 5-fluorouracil-treated cells did not (70).

Glioblastoma. Glioblastoma multiforme (GBM) is a primary malignant brain neoplasia that occurs in intracranial tissue or glial cells, which contribute to the supply of functional nutrients and oxygen to neurons (71). GBM patients typically have a poor prognosis, partly due to the infiltrative nature of glioma cells (72). Despite clinical and technological advances in the treatment of brain tumours over the last three decades, the survival of patients with GBM has not notably improved, and resistance to chemotherapy with agents such as temozolomide, is commonplace (73).

In an *in vitro* study, HC was shown to synergise with GTT, an isomer of vitamin E, to increase the death of glioma cells. This combined treatment activated caspase-3 by 7.1-79.0% and induced cell cycle arrest at the G2M and S-phases (74). In another study, the effect of HC and GTT in reducing the migration, invasion and colony formation of glioma cells was evidenced by a reduction in the downregulation of several genes in these pathways, namely cyclooxygenase (COX)-2, VEGFA, Jun and Wnt family member 5A (24). The ability of 1321N1, SW1783 and LN18 glioma cells to migrate was reduced by 16.1, 36.3 and 16.7%, respectively, when HC was combined with GTT (24,75). The ER-unfolded protein response (ER-UPR) pathway was postulated to contribute to the underlying mechanism for the HC and GTT combination. In addition, the expression of forkhead box protein M1, a crucial gene in pro-oncogenic signalling, was decreased in glioma by these combined treatments (24).

Liver cancer. Liver cancer was ranked as the sixth most common cancer in Malaysia and worldwide and the third most common cause of cancer-associated death in Malaysia in 2020 (51). The risk factors for liver cancer include hepatitis B virus, hepatitis C virus, fatty liver disease, alcohol-associated cirrhosis, smoking, obesity, diabetes, iron overload and various dietary exposures (76). Most patients with liver cancer have a poor prognosis, with only 5-15% of patients at an early stage

without cirrhosis being eligible for surgical excision (77). Patients with late-stage liver cancer are commonly treated with trans-arterial chemoembolisation (TACE) and the kinase inhibitor sorafenib as chemotherapy. As it the case with most chemotherapeutics, the long-term usage of TACE or sorafenib tends to cause side effects, toxicity and drug inefficacy (77).

A study revealed that the application of HC to HepG2 liver cancer cells pre-treated with BSO had cytotoxic effects, suggesting that the mechanism of action of HC was dependent on endogenous GSH. Although a low concentration of HC (12.5 μM) failed to reduce the viability of HepG2 cells when incubated for 24 h, 100 μM HC induced apoptosis over the same treatment period. These findings were confirmed by the observation of condensed chromatin by fluorescence microscopy and nuclear fragmentation using gel electrophoresis (78). It was concluded that these findings indicate that HC induces oxidative DNA damage and apoptosis in GSH-depleted HepG2 cells.

Oral cancer. Oral cancer is one of the most common head-and-neck cancers, which includes cancers of the lip, tongue, gum, palate, mouth, floor of the mouth, gingiva and other parts of the oral cavity (79). In 2020, India had the highest number of oral cancer cases and Malaysia was ranked sixth among Southeast Asian countries for the prevalence of this type of cancer (51). Oral cancer may occur due to infection with human papillomavirus (HPV), poor hygiene, poor dental care and the consumption of unhealthy food (79). The most common treatment for oral cancer is surgical resection alone or combined with radiotherapy or adjuvant therapy. Despite the advancement of surgical techniques, the number of cases of oral cancer in certain Asian regions continues to rise, partly due to habits including alcohol consumption, smoking, tobacco chewing and areca nut chewing, as well as the limited availability of tertiary healthcare for most of the population (80-85).

A study indicated that at concentrations of <0.1 mM, HC exhibited anti-oxidative properties, while at higher concentrations, it inhibited oral KB carcinoma cell growth and cell cycle progression (31). Furthermore, at concentrations of 10 and 50 μM HC acted as a scavenger of H_2O_2, reducing H_2O_2-induced chemiluminescence by 53 and 75%, respectively (31). In addition, 0.02 μM HC effectively scavenged superoxide created by xanthine/xanthine oxidase (31). HC was a more potent scavenger of superoxide than of H_2O_2. The treatment of oral KB cells with ≥0.1 mM HC for 24 h induced cell cycle arrest at the late S and G2/M phases and reduced the GSH levels of the cells. Interestingly, at the low concentration of 0.01 mM, HC inhibited the production of ROS in oral KB cells. However, the intracellular accumulation of ROS occurred at a higher concentration of HC (0.1 mM) (31). In another study, 50 and 100 μM HC effectively inhibited the expression of matrix metallopeptidase (MMP-9) induced by areca nut extract in squamous cell carcinoma of the tongue epithelial cells. Although these concentrations of HC inhibited the expression of MMP-9, which plays a vital role in cancer invasion and metastasis, it could not halt the proliferation of the cells (86).

In a study of the effect of HC on KB epithelial cells (87), exposure to HC at concentrations of 0.1 and 0.3 mM for

Table II. Anticancer activities of HC in various cancers based on *in vitro* and *in vivo* studies.

First author/s, year	Cancer type	Experimental models	Treatment	Major findings	(Refs.)
Paul et al, 2019	CML	K562-imatinib resistant	HC (2, 4 and 6 μg/ml) + TRAIL (200 ng/ml)	↑ROS, sensitised to TRAIL-induced apoptosis, ↑cytotoxic effect and induced potent anti-CML activity, induced caspase-8 cleavage, ↓XIAP and FLIP protein expression (4 and 6 μmol/l HC) via proteasomal degradation	(48)
Chakraborty et al, 2012	CML	Mouse xenografts with mutated or wild-type Bcr-Abl	HC (100 mg/kg)	↑Apoptosis, lifespan of tumour-bearing mice and ↓tumor growth	(25)
Chakraborty et al, 2012	CML	K562, KU812 and KCL22; K562	HC (0.5-20 μg/ml); HC (5 μg/ml)	↓Cell viability in a dose-dependent manner, ↑ROS, ↑NO, ↑apoptosis, no depletion of GSH; induced cleavage of caspase-9 and caspase-3, degraded PARP, induced JNK and eNOS phosphorylation (2.5 μg/ml)	(25)
Chakraborty et al, 2012	CML	Primary CML cells and leukaemic cells expressing mutated Bcr-Abl	HC (5 μg/ml)	↓Cell viability	(25)
Chowdhury et al, 2013	CML	K562	10 μM + 100 μM BSO	Disrupted the ROS balance and induced the production of RNS, cleaved PARP and caspase-3, triggered apoptosis-inducing factor-translocation, induced phosphorylation of JNK and ERK1/2, ↑iNOS	(49)
Chaudhari et al, 2014	CML	K562	HC (0, 5 and 15 μM) + curcumin (1, 2.5 and 5 μM)	↓Cell viability, activated mTOR, MAPK and mitochondrial signalling pathway, induced cleavage of caspase-3, caspase-9 and PARP, ↓Bad, Bax and BID protein expression, ↑phosphorylation of JNK1, p38, mTOR and S6K-1, and ↑4E-BP1 protein expression	(50)
Hemamalini et al, 2020	Breast cancer	Ehrlich ascites carcinoma mouse model	HC (200 and 400 mg/kg)	↓Tumour volume, ↑oxidative stress markers GSH, superoxide dismutase and catalase, ↓MDA levels, indicated to inhibit chemokine receptor type 4 via *in silico* receptor docking	(60)
Majumdar and Subramanian, 2019	Pancreatic cancer	MIA PaCa-2 and PANC-1	HC (25-100 μM)	↓Cell viability, ↓colony formation, ↑cell cycle arrest at G2/M, ↑mitotic catastrophe, induced EMT by ↓migration and invasion, ↓EMT-associated genes (Snail1, MMP9 and ICAM-1), ↓G2/M checkpoint protein cyclin B1 and CDC2 phosphorylation, ↑DNA response protein γH2AX and ↑phosphorylation of ATM and Chk1	(27)
Gundala et al, 2014	Prostate cancer	PC-3, C4-2, DU145 and 22Rv1; PC-3	IC$_{50}$ values 100, 35, 316 and 33 μM, respectively; 100 μM HC	↓Cell viability, ↑apoptosis, ↓clonogenicity, ↑ROS, ↑induced release of cytochrome *c* from mitochondria, inhibited cell cycle progression, ↑DNA damage, ↑cleavage of caspase-3 and PARP, ↑γH2AX, and ↑activation of autophagy protein markers LC3-IIB and beclin-1	(67)

Table II. Continued.

First author/s, year	Cancer type	Experimental models	Treatment	Major findings	(Refs.)
Gundala *et al*, 2014	Prostate cancer	PC-3–luc tumor xenografts in C47BL6/J mice	HC (150 mg/kg)	↓Tumour weight, ↑cleavage of caspase-3 and PARP	(67)
Rajedadram *et al*, 2021	Colorectal cancer	HT-29	HC (30 μg/ml)	↓Cell viability, ↑apoptosis, induced cell cycle arrest at G0/G1 and G2/M phases, induced activation of JNK and p38 MAPK	(70)
Rahman *et al*, 2019; Rahman *et al*, 2014	Glioblastoma	1321N1 (grade II); SW1783 (grade III); LN18 (grade IV)	12 μg/ml HC + 40 μg/ml GTT; 2 μg/ml HC + 40 μg/ml GTT; 29 μg/ml HC + 20 μg/ml GTT	↑Apoptosis, arrested cell cycle, ↓migration, invasion and colony formation, ↑ER stress pathway (XBP1, ATF4 and DDIT3), ↓FOXM1, E2F1 and PARP1, ↓cell cycle-associated genes CDC20 and CAV1, altered the splicing expression of RRAS2, SRSF3 and ADAMTS2	(24,74)
Rahman *et al*, 2022	Glioblastoma	1321N1 (grade II); SW1783 (grade III); LN18 (grade IV)	50 μg/ml EGCG + 20 μg/ml HC; 100 μg/ml EGCG + 25 μg/ml HC; 50 μg/ml EGCG + 10 μg/ml HC	↑Apoptosis, arrested cell cycle, ↓migration, invasion and colony formation, ↑apoptosis by activating caspase-3, regulation of ER-UPR activated inflammatory response pathway, ↓regulation of SEMA3A and SEMA3F, inhibited PLXNA1 and PLXNB2	(75)
Chen *et al*, 2000	Liver cancer	HepG2	100-200 μM HC, pretreated with 100 μM BSO	GSH dependent, ↑apoptosis, ↑DNA damage by 8-OH-dG formation, ↓cell viability; effect of HC was suppressed by catalase pretreatment	(78)
Chang, 2002	Oral cancer	Oral KB carcinoma cells	HC (10 μM-0.3 mM)	↓Cell viability, ↑apoptosis, ↑cell cycle arrest at late S and G2/M phases, ↑ROS, ↓reduced form of GSH, ↓adhesion to collagen type I and fibronectin at 100 and 250 μM HC	(31)
Chang *et al*, 2019	Oral cancer	SAS tongue cancer epithelial cells	HC (50 and 100 μM)	Inhibited ANE-induced MMP-9 production	(86)
Jeng *et al*, 2004	Oral cancer	KB epithelial cells	HC (0.1, 0.2 and 0.3 mM)	↑Cell cycle arrest at late S and G2/M phases, ↑ROS and ↓reduced form of GSH	(87)
Moushumi and Sumati V, 1994	Skin cancer	Female Swiss mice	HC (1 mg/day)	Restored normal DNA biosynthesis in skin exposed to DMBA	(92)

ADAMTS2, ADAM metallopeptidase with thrombospondin type 1 motif 2; ANE, areca nut extract; ATF4, activating transcription factor 4; BSO, buthionine sulfoximine; CAV1, caveolin 1; CDC2/20, cell division cycle 2/20; Chk1, checkpoint kinase 1; CML, chronic myelogenous leukaemia; DDIT3, DNA damage-inducible transcript 3; DMBA, dimethylbenz[a]anthracene; 4E-BP1, eukaryotic translation initiation factor 4E binding protein 1; E2F1, transcription factor 1; EGCG, epigallocatechin gallate; EMT, epithelial-mesenchymal transition; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulun; ERK, extracellular signal-regulated kinase; FLIP, FLICE-inhibitory protein; FOXM1, forkhead box protein M1; γH2AX, Ser-139-phosphorylated H2A histone family member X; GSH, glutathione; GTT, γ-tocotrienol; HC, hydroxychavicol; ICAM-1, intercellular adhesion molecule 1; JNK, c-Jun N-terminal kinase; LC3-IIb, light chain 3-IIb; 8-OH-dG, 8-hydroxy-2'-deoxyguanosine; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MMP, matrix metallopeptidase; mTOR, mammalian target of rapamycin; NO, nitric oxide; PARP, poly (ADP-ribose) polymerase; PLXNA1/B2, plexin A1/B2; RNS, reactive nitrogen species; ROS, reactive oxygen species; RRAS2, RAS related 2; S6K-1, S6 kinase 1; SEMA3A/F, semaphorin 3A/F; SRSF3, serine and arginine rich splicing factor 3; TRAIL, tumour-necrosis-factor-related-apoptosis-inducing ligand; UPR, unfolded protein response; XBP1, X-box binding protein 1; XIAP, X-linked inhibitor of apoptosis protein.

6 h resulted in S-phase arrest. However, when treated with 0.1 mM HC for 24 h, some KB cells progressed to the G2/M and G0/G1 phases, and when exposed to 0.3 mM HC for 24 h, KB cells were either arrested in the S phase or became apoptotic (87). HC also induced mitochondrial depolarisation in the cells as demonstrated by impaired rhodamine uptake. At a concentration of 0.3 mM, HC caused GSH depletion and the generation of intracellular ROS in KB cells, while at concentrations of 0.1 and 0.2 mM, HC raised the GSH levels of the KB cells (87).

Skin cancer. Skin cancer is a frequently occurring cancer for which sun exposure is the leading cause (88-90). Malignant melanoma (MM) and non-melanoma skin cancer (NMSC) are the two main types of skin cancer (88). Over the last 50 years, the incidence rates of both MM and NMSC have increased, with MM showing a 0.6% increase among adults (91).

In a study investigating the effect of HC on DNA biosynthesis in a female Swiss mice model of skin cancer, exposing the mice to 1 mg/day of HC demonstrated the ability to restore normal DNA biosynthesis in skin exposed to the carcinogen dimethylbenz[a]anthracene (92).

In summary, HC has shown anticancer effects in various cancer cell lines, including CML, glioma, breast, pancreatic, prostate, oral and colorectal cancer cells. In general, HC affects the JNK pathway, induces ROS, inhibits the cell cycle and lowers the viability of the cancer cells. However, data on the bioavailability of HC in cancer cells and animal models remains scarce. Bioavailability is the extent and rate at which an active compound enters the systemic circulation to reach the site of action (93). It remains unknown if HC goes directly to target sites or affects the organs, and the effects of HC in the body may be reduced as it migrates to the target site. Another aspect to consider is the inflammatory process that HC may induce in cancer cells. Unfortunately, little information is available on this in the literature, although the anti-inflammatory effects of HC have been studied in healthy cells. Table II summarises the anticancer activity of HC in various cancers based on *in vitro* and *in vivo* studies.

5. Anti-inflammatory activity of HC

Chronic inflammation is associated with cancer. Leukocytes and other phagocytic cells cause DNA damage in proliferating cells via the production of ROS and reactive nitrogen species to fight infection (94). The inflammatory process is mediated by two enzymes, namely COX and lipoxygenase (95). The COX and lipoxygenase cascades can lead to the development of multiple types of cancer (96,97).

In one study, HC decreased the generation of superoxide ions and the release of elastase by human neutrophils induced by the leukocyte chemotactic factor formyl-methionyl-leucyl-phenylalanine and the mycotoxin cytochalasin B (98). In another study, HC was shown to be a potent COX1/COX2 inhibitor, ROS scavenger and inhibitor of platelet calcium signalling, thromboxane B2 synthesis and aggregation (30). However, the anti-inflammatory effects of HC in the study were on non-cancer model organisms (30). In addition, 10, 50 and 100 μM HC was shown to elevate COX-2 expression in normal human oral keratinocytes in a concentration-dependent manner, increasing COX-2 mRNA expression by 2.81, 3.68 and 9.25 folds, respectively, when treated with HC for 18 h, and increasing COX-2 protein expression by 0.96, 2.81 and 4.09 folds, respectively compared with the untreated control (29).

Only a few studies have shown an anti-inflammatory effect of HC on malignant cells, and no specific molecular mechanism has been elucidated. For instance, when HC combined with epigallocatechin gallate was used to treat 1321N1 and LN18 glioma cells, the UPR pathway was induced, followed by activation of an inflammatory response. However, the molecular mechanism underlying the anti-inflammatory effect of HC was not elucidated (75). Future research may therefore focus on the anti-inflammatory properties of HC in cancer cells.

6. Conclusions and prospects

The efficacy of HC varies among cancers owing to different effects on pleiotropic pathways. Nevertheless, common pathways targeted by HC have been identified, namely the JNK, MAPK and eNOS signalling pathways. HC may exert its anticancer effect by altering proteins in apoptotic pathways, including caspase-3 and PARP. Overall, it appears that the anticancer potential of HC is dependent on the accumulation of ROS in cancer cells, which eventually lead to apoptotic cell death. However, despite numerous efforts to elucidate the physicochemical and biological characteristics of HC, various issues on bioavailability, potency and tissue and dose selectivity require clarification. Also, the adsorption of HC and the appropriate dosage remain uncertain due to limited studies using animal models. Therefore, future research should emphasise *in vivo* experimentation to ensure the safety and bioavailability of HC in humans. Furthermore, combining HC with other chemotherapeutics might be viable to determine whether a synergistic positive interaction occurs that facilitates the killing of cancer cells or sensitises them to chemotherapy.

Availability of data and materials

Not applicable.

Authors' contributions

NAM and AAR drafted the manuscript. SHAK reviewed and revised the manuscript content. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent of participants

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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