The Role of Herbal Medicine in Cholangiocarcinoma Control: A Systematic Review

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cholangiocarcinoma, herbal medicine, anticancer activity, *Atractylodes lancea* (Compositae), *Curcuma longa* (Zingiberaceae), *Garcinia hanburyi* (Clusiaceae), *Artemisia annua* (Compositae), *Zingiber officinale* (Zingiberaceae), *Andrographis paniculata* (Acanthaceae)

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ABSTRACT

The growing incidence of cholangiocarcinoma (bile duct cancer) and limited treatment options stimulate a pressing demand for research and the development of new chemotherapeutics against cholangiocarcinoma. This study aimed to systematically review herbs and herb-derived compounds or herbal formulations that have been investigated for their anti-cholangiocarcinoma potential. Systematic literature searches were conducted in three electronic databases: PubMed, ScienceDirect, and Scopus. One hundred and twenty-three research articles fulfilled the eligibility critera and were included in the analysis (68 herbs, isolated compounds and/or synthetic analogs, 9 herbal formulations, and 119 compounds that are commonly found in several plant species). The most investigated herbs were *Atractylodes lancea* (Thunb.) DC. (Compositae) and *Curcuma longa* L. (Zingiberaceae). Only *A. lancea* (Thunb.) DC. (Compositae) has undergone the full process of nonclinical and clinical development to deliver the final product for clinical use. The extracts of *A. lancea* (Thunb.) DC. (Compositae), *Garcinia hanburyi* Hook.f. (Clusiaceae), and *Piper nigrum* L. (Piperaceae) exhibit antiproliferative activities against human cholangiocarcinoma cells (IC_{50} < 15 µg/mL). Cucurbitacin B and triptolide are herbal isolated compounds that exhibit the most promising activities (IC_{50} < 1 µM). A series of experimental studies (*in vitro, in vivo*, and humans) confirmed the anti-cholangiocarcinoma potential and safety profile of *A. lancea* (Thunb.) DC. (Compositae) and its active compounds atractylodin and *β*-eudesmol, including the capsule pharmaceutical of the standardized *A. lancea* (Thunb.) DC. (Compositae) extract. Future research should be focused on the full development of the candidate herbs to deliver products that are safe and effective for cholangiocarcinoma control.

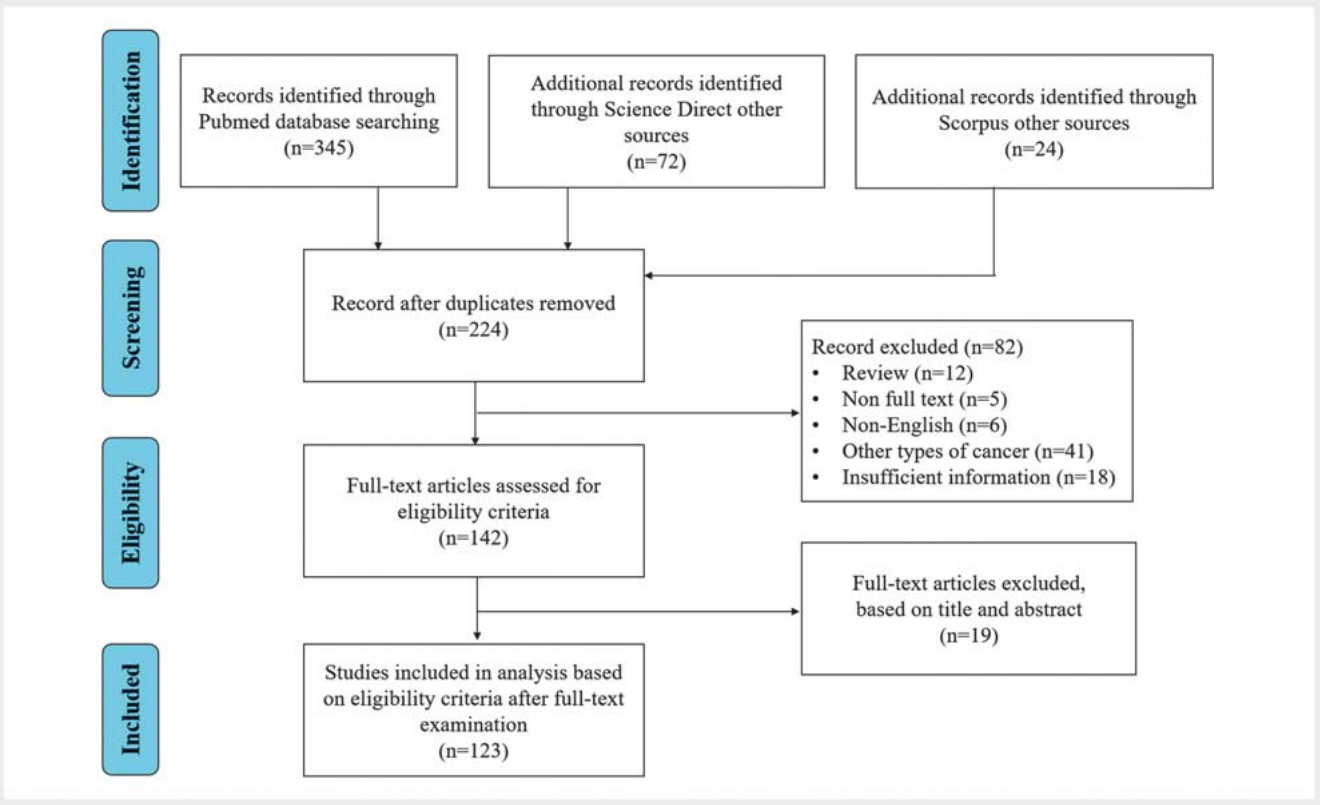
ABBREVIATIONS

5-FU	5-fluorouracil
ADP	adenosine diphosphate
AKT	protein kinase B
ALNP	atractylodin-loaded PLGA nanoparticle
AMPK	5′ AMP-activated protein kinase
AP1	activator protein 1
Apaf-1	apoptotic protease-activating factor 1
AT	atractylodin
Bax	Bcl2-associated X protein
Bcl2	B-cell lymphoma 2
BE	beta-eudesmol
BID	twice per day
CAF	cancer-associated fibroblast
CCA	cholangiocarcinoma
Cdk	cyclin-dependent kinase
CHOP	C/EBP homologues protein
cIAP	cellular inhibitor of apoptosis protein
COVID-19	coronavirus disease of 2019
COX2	cyclooxygenase 2
DAPK1	death-associated protein kinase 1
DMN	dimethylnitrosamine
DR	death receptors
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
eIF2α	eukaryotic initiation factor 2
E_{max}	maximum drug effect
EMSA	electrophoresis mobility shift assay
EPMC	ethyl-*p*-methoxycinnamate
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
GLI1	glioma-associated oncogene homologue 1
GSKβ	glycogen synthase kinase beta
HO1	heme oxygenase 1
HS	hinesol
ICAM1	intercellular adhesion molecule 1
IFNγ	interferon gamma
IL6	interleukin 6
JAK	Janus kinase
JNK	Jun N-terminal kinase
MAPK	mitogen-activated protein kinase
MCL-1	myeloid cell leukemia-1
MDR	multidrug resistance
MMP	matrix metalloproteinases
MRP	multidrug resistance associated protein
MTD	maximum tolerated dose
mTOR	mammalian target of rapamycin
NFκB	nuclear factor kappa light chain enhancer of activated B cells
NK	natural killer
OV	Opisthorchis viverrini
p38	38-kilodalton protein kinase
PBMC	peripheral blood mononuclear cell
PI3K	phosphoinositide 3-kinase
PLGA	poly lactic-co-glycolic acid
RAS	rat sarcoma
Rb	retinoblastoma
ROS	reactive oxygen species
RT-PCR	real-time PCR
SRB	sulphorhodamine B
STAT	signal transducer and activator of transcription
TEM	transmission electron microscope
TR1	type 1 regulatory T cells
TRAF1	tumor necrosis factor receptor associated factor 1
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
VEGFR	vascular endothelial growth factor receptor
WST	water-soluble tetrazolium salts
XIAP	X-linked inhibitor of apoptosis protein

Introduction

CCA is a malignant bile duct cancer of epithelial cells with high morbidity and mortality. The world's highest incidence is reported from the northeastern part of Thailand, with an age-standardized incidence rate of 33.4 per 100 000 in males and 12.3 per 100 000 in females. It is the second most common hepatic malignancy in the world after hepatocellular CCA. Increasing incidence and mortality from CCA have been reported globally [1]. Several risk factors are associated with CCA development, including primary sclerosing cholangitis, cirrhosis, fibropolycystic hepatic disease, hepatolithiasis, congenital intrahepatic biliary stones, viral hepatitis, and liver fluke infection (*Opisthorchis viverrini* and *Clonorchis sinensis*). Infection with *O. viverrini* is a risk factor for almost all cases of CCA in Thailand [2]. Treatment and control of CCA remain unsatisfactory due to the lack of sensitive and specific diagnostic tools for early detection, as well as effective drugs. The overall 5-year survival rate of CCA patients is less than 5%. Surgical resection is the curative treatment option eligible for patients with an early-stage tumor. Gemcitabine and/or cisplatin-based chemotherapy is the first-line treatment option for patients with advanced or metastatic disease. However, the effectiveness of these drugs is limited, with the median overall survival of less than 1 year [3]. The growing incidence of CCA and limited treatment options hasten a pressing demand for research and development of new chemotherapeutics against CCA.

In recent years, natural products and the research and development of herbs for cancer chemotherapy have been an intensive area of research. This is due to the diverse chemical structures and bioactivities of herbs that could be exploited as promising drug candidates for various types of cancer. Numerous studies have been carried out to discover effective cancer chemotherapeutic agents from plant sources with low toxicity. Examples of successful drugs for cancer include vincristine, vinblastine, etoposide, teniposide, paclitaxel, vinorelbine, docetaxel, topotecan, camptothecin, and irinotecan [4]. The aim of this study was to systematically review herbs and herb-derived compounds that have been investigated for their anti-CCA potential both *in vitro, in vivo*, and humans. Information obtained was analyzed to facilitate further



▶ **Fig. 1** Flow chart of the article selection process.

development of effective and safe anti-CCA drugs in a systematic approach.

Results and Discussion

A total of 224 articles from PubMed, ScienceDirect, and Scopus databases were downloaded to the EndNote database. Eighty-two articles were excluded, and further analysis of the titles and abstracts of the remaining 142 articles led to the exclusion of 19 articles (excluded based on title and abstract). Finally, 123 articles were included in the analysis. The flow diagram of the study inclusion and exclusion is presented in ▶ **Fig. 1**. Antiproliferative activities of plant extracts or active compounds are summarized in ▶ **Table 1** and results of clinical studies of some herbal formulations are summarized in ▶ **Table 2**. Mechanisms of antiproliferative activities including *in vivo* studies in animals are provided in the Supporting Information. The included articles involve 68 herbs, isolated compounds, and/or synthetic analogs, 9 herbal formulations, and 199 compounds that are commonly found in several plant species. The most investigated plant was *Atractylodes lancea* (Thunb.) DC. (Compositae) (n = 17), followed by *Curcuma longa* L. (Zingiberaceae) (n = 15), *Garcinia hanburyi* Hook.f. (Clusiaceae) (n = 6), *Artemisia annua* L. (Compositae) (n = 5), *Zingiber officinale* Roscoe (Zingiberaceae) (n = 5), *Andrographis paniculata* (Burm.f.) Nees (Acanthaceae) (n = 4), *Capsicum* spp. (Solanaceae) (n = 3), *Derris indica* (Lamk.) Benn. (Leguminosae) (n = 3), *Piper longum* L. (Piperaceae) (n = 3), and *Tripterygium wilfordii* Hook. f. (Celastraceae) (n = 3). Other plants were reported in one or two research articles. Pra-Sa-Prao-Yai was the most investigated formulation (n = 2). Resveratrol (n = 5) and capsaicin (n = 3) derived from several plants was the most investigated compounds for anticancer activity against CCA. Most studies reported the antiproliferative activities using different *in vitro* tests (n = 108), including MTT, SRB, WST-1, Hoechst, neutral red, acridine orange/ethidium bromide, cell counting kit-8, crystal violet, PrestoBlue, calcein-AM, tryphan blue, cell titer 96 aqueous, IncuCyte zoom, morphological examination, flow cytometry, and clonogenic assays. *In vivo* evaluation of anti-CCA activity in animal models [xenograft mouse model, OV/DMN-induced CCA hamster model, and allograft hamster model] was reported in 26 articles. Mechanisms or targets of action at the molecular or cellular level were reported in 95 studies. Others involved studies on antioxidative (n = 3) and immunomodulatory activities (n = 2), as well as their inhibitory activities on cell migration (n = 22) and cell invasion (n = 17), pharmacokinetic studies (n = 2), clinical studies (safety and/or efficacy) (n = 3), development of nanoformulations (n = 2), and synergizing effects on chemotherapeutic drugs (n = 5).

The potential role of herbs/herbal medicines for CCA control has been one of the focuses in CCA research, as seen by a relatively large number of research articles published during the years 2000 to 2021. Evidence-based knowledge is provided by scientific support from *in vitro, in vivo*, and clinical studies in a total of 68 herbs, 9 herbal formulations, and 199 isolated compounds or syn-

▶ **Table 1** Plants/isolated compounds/symthetc compounds (underlined) under investigation and available antiproliferative activity against CCA cell lines.

References	Plants/Active compounds (Family)	Antiproliferative activity
[5]	Crude ethanol extracts: *Amomum testaceum* Ridl. (Zingiberaceae), *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. (Apiaceae), *Angelica sinensis* (Oliv.) Diels. (Apiaceae), *Anethum graveolens* L. (Apiaceae), *Artemisia annua* L. (Compositae), *Asclepias curassavica* L. (Apocynaceae), *Atractylodes lancea* (Thunb.) DC. (Compositae), *Cuminum cyminum* L. (Apiaceae), *Curcuma longa* L. (Zingiberaceae), *Dioscorea membranacea* Pierre ex Prain & Burkill (Dioscoreaceae), *Dracaena loureirin* Gagnep. (Asparagaceae), Foeniculum vulgare Mill. (Apiaceae), *Kaempferia galanga* L. (Zingiberaceae), Ligusticum sinense Oliv. (Apiaceae), *Mammea siamensis* Kosterm. (Guttiferae), *Mesua ferrea* L. (Calophyllaceae), *Mimusops elengi* L. (Sapotaceae), *Myristica fragrans* Houtt. (Myristicaceae), *Nigella sativa* L. (Ranunculaceae), *Piper chaba* Hunt. (Piperaceae), *Piper interruptum* Opiz. (Piperaceae), *Piper sarmentosum* Roxb. (Piperaceae), *Plumbago indica* L. (Plumbaginaceae), *Smilax corbularia* Kunth (Smilacaceae), *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae), *Zingiber officinale* Roscoe (Zingiberaceae), *Zingiber ligulatum* Roxb. (Zingiberaceae), Ben-ja-Kul 1 formulation, Ben-ja-Kul 2 formulation, Pra-Sa-Prao-Yhai formulation, Tein-5 formulation	*A. lancea* (Thunb.) DC. (Compositae): most potent and selective against CL6 cells (IC_{50} = 24.09 µg/mL, SI = 8.6); five others with promising activity (< 50 % cell survival at 50 µg/mL) = *K. galanga* L. (Zingiberaceae), *Z. officinal* Roscoe (Zingiberaceae), *P. chaba* Hunt. (Piperaceae), *M. ferrea* L. (Calophyllaceae), and Pra-Sa-Prao-Yhai formulation (IC_{50}s of 37.36, 34.26, 40.74, 48.23, 44.12 µg/mL, respectively)
[33]	*Cardiospermum halicacabum* L. (Sapindaceae), *Gomphrena celosioides* Mart. (Amaranthaceae), *Scoparia dulcis* L. (Plantaginaceae) (ethanolic extracts)	*S. dulcis* L. (Plantaginaceae): most potent (56–75 % growth inhibition on KKU-100 and KKI-213 cells at 250 µg/mL for 72 h)
[17]	*Andrographis paniculata* (Burm.f.) Nees (Acanthaceae)/Semisynthetic andrographolide analog (19-triphenylmethyl ether andrographolide, AG 050)	Excellent activity against KKU-M213 cells (IC_{50} = 3.33 µM)
[18]	*Andrographis paniculata* (Burm.f.) Nees (Acanthaceae)/14-deoxy-11,12-didehydroandrographolide analogs	Analogs 5a, 5b: most potent and selective against KKU-M213 cells (IC_{50} = 3.37, 3.08 µM); KKU-100 = 2.93, 3.27 µM
[19]	*Andrographis paniculata* (Burm.f.) Nees (Acanthaceae)/Andrographolide	Significant activity against KKU-100 cells (IC_{50} ~ 120 µM)
[34]	*Aesculus hippocastanum* L. (Sapindaceae)/*β*-escin	IC_{50} Mz-ChA1 cells: 34.21 µM (24 h), 28.48 µM (48 h), 22.1 µM (72 h); SK-ChA1 cells: 59.04 µM (24 h), 41.69 µM (48 h), 33.3 µM (72 h); QBC939 cells: 63.3 µM (24 h), 44.36 µM (48 h), 34.06 µM (72 h)
[35]	Anthocyanin complex [from cobs of purple way corn Zeamays, certina Kulesh, and petals of blue butterfly pes *Clitoria ternatea* L. (Leguminosae)]	IC_{50} for KKU-213 cells = 620 µg/mL
[36]	*Arachis hypogaea* L. (Leguminosae)/Peanut testa extract, KK4 and ICG15042	Potent activity against KKU-M214 cells (KK4: IC_{50} = 38.28 µg/mL; ICG15042: IC_{50} = 43.91 µg/mL) and KKU-100 cells: (KK4: IC_{50} = 78.40 µg/mL; ICG15042: IC_{50} = 82.77 µg/mL) at 72 h
[37]	*Artemisia annua* L. (Compositae)/Artemisinins	Potent activity against CL6 cells: IC_{50} = 339 µM (artemisinin), 131 µM (artesunate), 354 µM (*β*-artemeter), 75 µM (dihydroartemisinin)

continued

▶ **Table 1** *Continued*

References	Plants/Active compounds (Family)	Antiproliferative activity
[21]	*Atalantia monophylla* DC. (Rutaceae)/7 new benzoyltyramines, atalantums A–G (1–7) and 5 known compounds	Compound **5**: most potent activity against KKU-M156 (IC_{50} = 1.97 µM), 4.7-fold higher than ellipticine standard. Compound **1**: potent activity against KKU-M214 (IC_{50} = 3.06 µM), comparable with 5-FU. Compounds **2, 4, 11**: more potent activity against KKU-M213 than ellipticine (IC_{50} = 2.36, 5.63, 2.71 µM). Compounds **1, 5, 7**: activity against KKU-M214 (IC_{50} = 3.06, 8.44, 7.37 µM, respectively).
[22]	*Atalantia monophylla* DC. (Rutaceae)/limonophyllines A–C (1, 4, 5), limonoids (2, 3), acridone alkaloids (6–16)	Compounds **12, 14, 16**: activity against KKU-M156 cells (IC_{50} = 3.39–4.1 µg/mL)
[38]	*Atractylodes lancea* (Thunb.) DC. (Compositae)/Atractylodin	IC_{50} = 216.8 µM for CL6 cells
[39]	*Atractylodes lancea* (Thunb.) DC. (Compositae)/Atractylodin (AT) and Atractylodin-loaded PLGA nanoparticle (ALNPs)	IC_{50} for CL6 cells, ALNPs vs. AT: 29.28 vs. 56.36 µM (24 h), 35.06 vs. 37.66 µM (48 h), 50.74 vs. 52.02 µM (72 h) µg/mL; IC_{50} for HuCC-T1 cells: ALNPs vs. AT: 47.68 vs. 53.66 µg/mL (24 h), 66.09 vs. 59.74 µM (48 h), 71.3 vs. 76.15 µg/mL (72 h)
[40]	*Atractylodes lancea* (Thunb.) DC. (Compositae)/Atractylodin (AT) Atractylodin-loaded PLGA nanoparticle (ALNPs)	IC_{50} for CL-6 cells: ALNPs vs. AT: 15 vs. 43 (24 h), 23 vs. 40 (48 h), 43 vs. 40 (72 h) µg/mL; IC_{50} for HuCC-T1 cells: ALNPs-1 vs. AT: 9 vs. 65 (24 h), 16 vs. 42 (48 h), 39 vs. 65 (72 h) µg/mL
[41]	*Atractylodes lancea* (Thunb.) DC. (Compositae)/Atractylodin and *β*-eudesmol	IC_{50} for CL6 cells: atractylodin = 41.66 µg/mL, *β*-eudesmol = 39.33 µg/mL
[42]	*Atractylodes lancea* (Thunb.) DC. (Compositae)/*β*-eudesmol	IC_{50} for CL6 cells = 166 µM
[43]	*Caesalpinia mimosoides* Lam. (Leguminosae) ethylacetate extract/ Gallic acid (natural: nGA, commercial: cGA)	IC_{50}: nGA = 120 µM (M213 cells) and 124 µM (M214 cells cGA), 119 µM (M213 cells), and 147 µM (M214 cells)
[44]	*Clausena harmandiana* (Pierre) Pierre ex Guillaumin (Rutaceae) hexane, ethyl acetate, methanol extracts/isolated and purified 12 azarbazoles and coumarins	7-hydroxy-heptaphylline and nordentatin: potent activity against KKU-OCA17 cells (IC_{50} = 88.7, 46.1 µM, respectively and KKU-214 cells (IC_{50} = 43.7, 39.1 µM, respectively)
[45]	Corilagin (natural plant polyphenol tannic acid)	IC_{50} for QBC9939 and MZ-Cha-1 cells = 39.73 and 36.88 µM, respectively
[46]	*Cratoxylum formosum* (Jack) Benth. & Hook. f. ex Dyer (Hypericaceae) aqueous and ethanolic Dyer leaf extract	Potent activity (IC_{50} for the aqueuous extract = 11.3 µg/mL, ethanol extract = 12.1 µg/mL)
[24]	*Curcuma longa* L. (Zingiberaceae)/Curcumin	IC_{50} = 5–17 µM (sensitive) for KKU-100, KKU-214, and KKU-OCA17 cells
[25]	*Curcuma longa* L. (Zingiberaceae)/Curcumin	IC_{50} = 5.9 µM for KKU-214 cells
[26]	*Curcuma longa* L. (Zingiberaceae)/Curcumin	Activity against CCLP-1 cells (10, 48, and 56% growth inhibition) and SG-231 cells (13, 25, and 50%) at 7.5, 10, and 15 mM, repectively
[27]	*Curcuma longa* L. (Zingiberaceae) New allylated mono-carbonyl curcumin analogs (MACs)	Compound **6c**: potent activity (IC_{50} for HuCCA cells = 8.7 µM, QBC-939 cells = 9.3 µM, and RBE = 8.9 µM)
[31]	*Derris indica* (Lamk.) Benn. (Leguminosae)/Candidione	Potent activity against KKU-M156 cells: IC_{50} = 6 µg/mL (17 µM) and 4.24 µg/mL (12.03 µM) at 8 and 24 h, respectively; KKU-M213 cells: IC_{50} = 5.7 µg/mL (16.17 µM) and 5.74 µg/mL (15.28 µM) at 8 and 24 h, respectively
[47]	*Derris indica* (Lamk.) Benn. (Leguminosae) ethylacetate extract/ a new furanoflavonoid derivative, 4′-hydroxypinnatin (1) and 5 known compounds	Pinnatin: potent activity against KKU-100 cells (IC_{50} = 6.0 µg/mL), E_{max} of 88–90% Flavone 5: highest activity against KKU-100 cells (IC_{50} = 1.3 µg/mL), but with moderate efficacy (E_{max} of 50.7%)
[48]	*Derris indica* (Lamk.) Benn. (Leguminosae) hexane extract/isolated Derrivanone (1) and Derrischalcone + 14 known compounds	Potent activity against KKU-M156 cells: Chalcones 2, 3, 4: IC_{50} = 7.0, 0.73, 0.59 µg/mL, respectively; Flavanones 14, 15, 16: IC_{50} = 0.59, 7.8, 2.4 µg/mL, respectively
[49]	*Derris malaccensis* (Benth.) Prain (Leguminosae)/Pomiferin (prenylated isoflavonoid)	IC_{50} for HuCCA-1 cells = 0.9 µg/mL

continued

▶ **Table 1** *Continued*

References	Plants/Active compounds (Family)	Antiproliferative activity
[50]	*Derris malaccensis* (Benth.) Prain (Leguminosae)/Pomiferin-4′-O-methyl ether, and a new prenylated chalcone, 2′,4′-dihydroxy-4-methoxy-3′-(2-hydroxy-3-methylbut-3-enyl)chalcone, 4 known flavonoids	Compounds **2** and **3**: potent activity against HuCCA-1 cells (IC_{50} = 4.8 and 3.8 µg/mL, respectively) Compounds **1, 4, 5, 6**: weak activity against HuCCT-1 cells (IC_{50} = 10.5, 14.0, 24.0, and 25.0 µg/mL, respectively)
[8]	*Dioscorea membranacea* Pierre ex Prain & Burkill (Dioscoreaceae) ethanol extract/7 isolated compounds	Crude extract: weak but selective activity against KKU-M156 cells (IC_{50} = 30.49 µg/mL); Compound **5**: selective activity against KKU-M156 cells (IC_{50} = 3.46 µM); Compounds **1**–**3**: no activity against KKU-156 cells (IC_{50} = 4100 µM)
[6]	*Garcinia hanburyi* Hook.f. (Clusiaceae) ethyl acetate and methanol extracts & fractions	Ethyl acetate extracts from bark (VR12874) and fruits (VR11626): potent activity (IC_{50} = 1.84–2.49 and 1.69–4.41 µg/mL); VR12876 and VR12879: weak activity; VR12880: no activity
[51]	*Garcinia hanburyi* Hook.f. (Clusiaceae)/4 caged xanthones: isomorellin, isomorellinol, forbesione gambogic acid	IC_{50}: Isomorellin: KKU-100 cells = 0.11 µM, KKU-156 cells = 0.12 µM; Isomorellinol: KKU-100 cells = 2.2 µM, KKU-M156 cells = 0.43 µM; Forbesione: KKU-100 cells = 0.15 µM, KKU-M156 cells = 0.02 µM; Gambogic acid: KKU-100 cells = 2.64 µM, KKU-M156 cells = 0.03 µM)
[52]	*Garcinia hanburyi* Hook.f. (Clusiaceae)/isomorellin	IC_{50} for KKU-100 cells vs. KKU-M156 cells: 6.2 vs. 1.9 µM (24 h), 5.1 vs. 1.7 µM (48 h), 3.5 vs. 1.5 µM (72 h)
[53]	*Holothuria scabra* Jaeger (sea cucumber)/Scabraside D (sulfated triterpene glycoside)	Significant activity against CL6 cells (IC_{50} = 12.8 µg/mL at 24 h)
[9]	*Kaempferia galanga* L. (Zingiberaceae) ethanol extract/Ethyl-p-methoxycinnamate (EPMC)	Extract and EPMC: moderate activity against CL6 cells (IC_{50} = 64.2, 49.19 µg/mL; SI = 2.2, 2.09)
[10]	*Kaempferia galanga* L. (Zingiberaceae) ethanol extract/Ethyl-p-methoxycinnamate (EPMC)	Moderate activity against CL6 cells: extract IC_{50} for CL6 cells = 78.41 µg/mL, SI = 4.44; EPMC: IC_{50} = 100.76 µg/mL, SI = 2.2; moderate activity against HuCCT1 cells: extract IC_{50} = 66.03 µg/mL, SI = 6.04; EPMC IC_{50} = 156.6 µg/mL, SI = 2.23
[54]	*Kaempferia parviflora* Wall. ex Baker (Zingiberaceae) (crude ethanol extract)/5,7,4- trimethoxyflavone (KP.8.10)	Flavonoid component in *K. parviflora* Wall. Ex Baker extract (KP.8.10): potent activity against HuCCA1 cells (IC_{50} = 46.1 µg/mL) and RMCCA-1 cells (IC_{50} = 62 µg/mL)
[20]	*Mylabris phalerata* (Pallas) or *Mylabris cichorii* (Laeus)/Cantharidin, Norcantharidin	Cantharidin: most sensitive (IC_{50}: RBE cells = 2 µM, QBC939 cells = 3 µM, HCCC9810 cells = 3 µM)
[55]	Phenformin and Quercetin and Myricetin (from several plant species)	Quercetin: enhancement of activity of phenformin against KKU-256 cells (IC_{50} = 1363 µM)
[56]	*Phomopsis archeri* B. Sutton (*fungus*)/phomoarcherins A–C (sesquiterpenes), kampanol A, R-mevalonolactone, ergosterol, ergosterol peroxide	Compounds **1**–**4**: IC_{50} = 0.1–19.6 µg/mL (KKU-100, KKU-M139, KKU-M156, KKU-M213, and KKU-M214 cells)
[57]	*Pinellia ternata* (Thunb.) Makino (Araceae/Banxia: polysaccharide (PTPA)	Sk-ChA-1 cells: most sensitive (IC_{50}: SNU-245, CL-6, Sk-ChA-1, and MZ-ChA-1 cells = 194, 76.9, 57.2, and 29.2 mg/mL, respectively)
[28]	*Piper longum* L. (Piperaceae)/Piperlongumine	IC_{50} for KKU-055, KKU-213, KKU-214, KKU-139, KKU-100, MMNK1, and NIH3T3 cells = 4.2, 5.2, 6.2, 8.8, 15.9, 5.7 and, 12.7 µM, respectively
[29]	*Piper longum* L. (Piperaceae)/Piperlongumine	IC_{50} for HuCCT-1–1 cells = 24.8 and 4.2 µM at 24 and 48 h, respectively
[7]	*Piper nigrum* L. (Piperaceae)/Piperine, Piperine-free *Piper nigrum* (black pepper) dichloroqmethane extract (PFPE)	PFPE: most potent and selective, especially on KKU-M213 cells (IC_{50} = 13.70 µg/mL) and TFK-1 cells (IC_{50} = 15.30 µg/mL)
[58]	*Pistacia atlantica* Desf. (Anacardiaceae)/Mastic gum resin	Activity against KMBC cells: IC_{50} = 15.34 µg/mL
[32]	*Plumbago indica* L. (Plumbaginaceae)/Plumbagin	IC_{50} for CL6 cells = 24.00 µM, SI = 2.28 (low)

continued

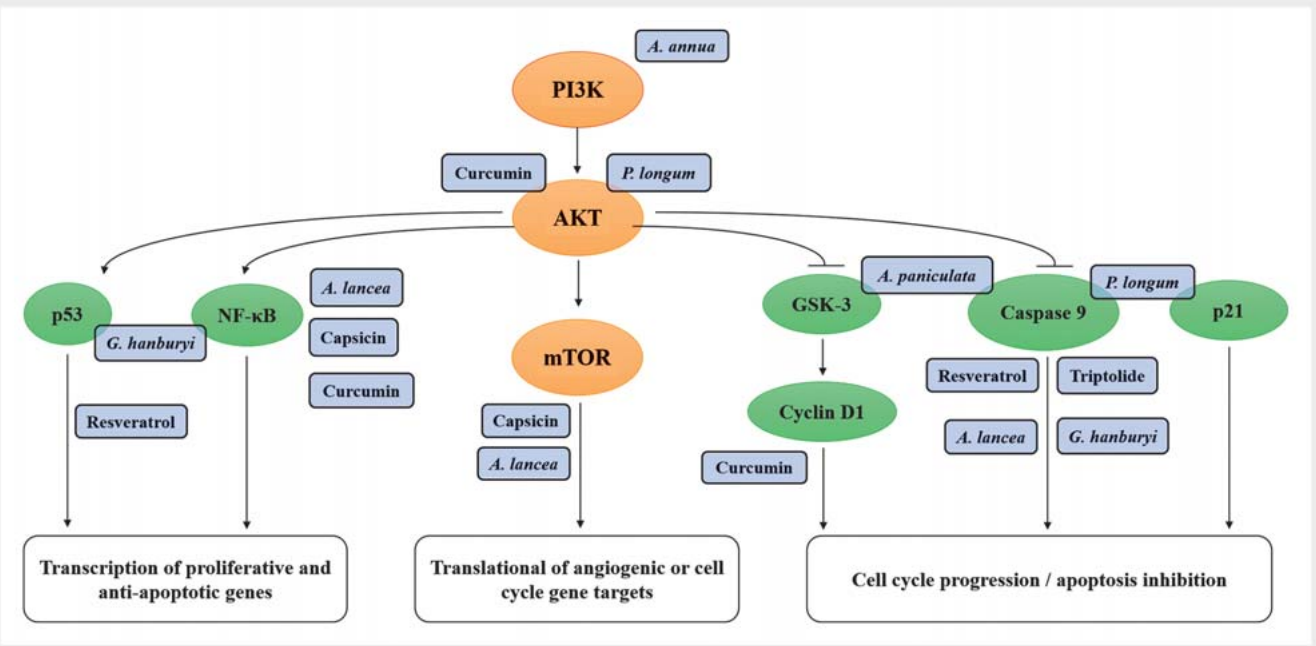
▶ **Table 1** *Continued*

References	Plants/Active compounds (Family)	Antiproliferative activity
[30]	*Reseda luteola* L. (Resedaceae)/Luteolin	Potent activity against KKU-M156 cells (IC_{50} = 10.5 and 8.7 µM at 24 and 48 h, respectively)
[16]	*Rhinacanthus nasutus* (L.) Kurz (Acanthaceae)/Rhinacanthin-C	Potent activity against KKU-M256 cells (IC_{50} = 1.50 µM)
[59]	*Tanacetum parthenium* L. (Compositae)/Parthenolide	IC_{50} for SCK cells = 10 µM
[23]	*Tiliacora triandra* (Colebr.) Diels (Menispermaceae)/Tiliacorinine	Significant activity against KKU-M055, KKU-100, KKU-M213, and KKU-M214 cells (IC_{50} = 4.5–7 µM)
[13]	*Trichosanthes cucumerina* L. (Cucurbitaceae)/Cucurbitacin B (CuB) (natural tetracyclic triterpene)	Potent activity against KKU-213: IC_{50} = 0.048 µM (24 h), 0.036 µM (48 h), and 0.032 µM (72 h) µM; KKU-214: IC_{50} = 0.088 µM (24 h), 0.053 µM (48 h), and 0.04 µM (72 h)
[14]	*Tripterygium wilfordii* Hook. f. (Celastraceae)/Triptolide	Potent activity against HaLCCA-1.1, HaLcca-2, HaTCCA-1.1 cells (IC_{50} = 0.05 mg/mL for all cells)
[15]	*Tripterygium wilfordii* Hook. f. (Celastraceae)/Triptolide	IC_{50} for HuCCT1, QBC939, and FRH0201 cells = 12.6, 20.5, 18.5 nM at 48 h, respectively
[11]	*Zingiber officinale* Roscoe (ginger) (Zingiberaceae) ethanol extract	Promising activity against CL6 cells (IC_{50} for each assay = 10.95, 53.15 µg/mL, SI = 18.09, 3.19)

SI = selectivity index

▶ **Table 2** Clinical studies of potential herbs and herbal formulations for CCA.

Ref	Plants/Active compounds	Methodology	Key findings
[124]	*Atractylodes lancea* (Thunb.) DC. (Compositae) ethanol standardized extract (CMC capsule formulation)	Clinical study: Phase I study, 48 healthy participants. Thais: *Group 1*: single oral dose of 1000 mg of *A. lancea* or placebo (20 : 4 participants). *Group 2*: daily oral doses of 1000 mg *A. lancea* or placebo daily for 21 days (20 : 4 participants). Clinical parameters: assessment of safety and tolerability. Pharmacokinetics: model-dependent and model-independent analysis.	Well tolerated in both groups. Atractylodin: rapidly absorbed but with low systemic exposure and residence time. No difference in the pharmacokinetics following a single or multiple dosing, suggesting the absence of accumulation and dose dependency in human plasma after continuous dosing for 21 days.
[137]	*Atractylodes lancea* (Thunb.) DC. (Compositae) ethanol standardized extract (CMC formulation)/β-eudesmol and atractylodin	Antiproliferation of PBMCs against CCA (CL6) (flow cytometry-based NY cytotoxic assay). Clinical study: Phase I study, 48 healthy participants. Thais receiving a single (1000 mg) or multiple oral dosing (1000 mg for 21 days) or placebo. Immunomodulation: cytokine levels (cytokine bead assay) and expression (RT-PCR); lymphocyte subpopulations (flow cytometry).	Immunomodulatory activity of *A. lancea* (Thunb.) DC. and compounds in complement with the direct action on apoptosis induction. Atractylodin: significant inhibition of IL6, TNF-α; *A. lancea* at a single dose: suppression of IFNγ and IL10, increase of B cells, increase of NK, CD4+, CD8+ cells, and a trend of increased antiproliferation activity of PBMCs at 24 h. *A. lancea* (Thunb.) DC. at multiple dosing: suppression of all cytokine production, increase of CD4+ and CD8+, increase of antiproliferative activity of PBMCs at 24 h (terminated at 48 h of dosing).
[155]	PHY906 formulation	Clinical study: open-label phase I trial (800 mg BID on days 1–4 + escalating doses of capecitabine (1000, 1250, 1500, 1750 mg/m^2), orally twice daily on days 1–7 of a 14-day cycle (7/7 schedule) in CCA (n = 1), pancreatic cancer (n = 15), colon cancer (n = 6), esophageal cancer (n = 1), unknown primary cancer (n = 1).	Well-tolerated at MTD of 1500 mg/m^2 BID administered in a 7/7 schedule, in combination with PHY906 800 mg BID on days 1–4; partial response (n = 1), stable disease > 6 weeks (n = 13).



▶ **Fig. 2** Proposed molecular targets and signaling pathways of potential herbs and isolated compounds/synthetic analogs on human CCA.

thetic analogs. The plants that were investigated the most were *A. lancea* (Thunb.) DC. and *C. longa* L. Other plants with more than three research articles published on antiproliferative activities included *G. hanburyi* Hook.f., *A. annua* L., *Z. officinale* Roscoe, and *A. paniculata* (Burm.f.) Nees. The previously reported studies of various potential herbs (extracts or isolated compounds/synthelic analogs) for CCA focused on their antiproliferative activities against CCA cell lines or antitumor activities in animal models, activities on cell invasion and migration, and underlying mechanisms or targets of their actions [5–155]. None of these herbs/isolated compounds/synthetic analogs, except *A. lancea* (Thunb.) DC., has undergone the full process of nonclinical, clinical, and pharmaceutical development to deliver final products for clinical use. The IC_{50} (concentration that inhibits cell growth by 50 %) values indicating the potency of activities were not reported for most herbs/isolated compounds/synthetic analogs/herbal formulations investigated. The potency of activity of the antiproliferative activity against human CCA cells was classified according to the IC_{50} as (i) weak activity (IC_{50} > 100 µg/mL for the herbal extract and > 100 µM for the isolated compounds/synthetic analogs), (ii) moderate activity (IC_{50} 10–100 µg/mL for the herbal extract and 10–100 µM for the isolated compounds/synthetic analogs), and (iii) relatively potent (IC_{50} < 10 µg/mL for the herbal extract and < 10 µM for the isolated compounds/synthetic analogs). Based on available published data, the antiproliferative activities of the extracts of *A. lancea* (Thunb.) DC., *G. hanburyi* Hook.f., and *Piper nigrum* L. (Piperaceae) are classified as potent [5–7], while those of *Dioscorea membranacea* Pierre ex Prain & Burkill (Dioscoreaceae), *Kaempferia galanga* L. (Zingiberaceae), *Mesua ferrea* L. (Calophyllaceae), *Piper chaba* Hunt. (Piperaceae), *Z. officinale* Roscoe, and Pra-Sa-Prao-Yhai formulation are classified as moderate [5, 8–11], and that of sho-saiko-to is classified as weak activity [12]. For the isolated compounds/synthetic analogs, those with the most potent activity are cucurbitacin B and triptolide [from *T. wilfordii* Hook. f.: IC_{50} < 1 µM] [13–15], followed by rhinacanthin C [from *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae): IC_{50} = 1.5 µM] [16], compounds from *D. membranacea* Pierre ex Prain & Burkill: IC_{50} = 1–2 µM [8], andrographolide and analogs [from *A. paniculata* (Burm.f.) Nees: IC_{50} = 3 µM] [17–19], cantharidin and norcantharidin [from *Mylabris phalerata* (Pallas): IC_{50} = 2–3 µM] [20], isolated/synthetic compounds from *Atalantia monophylla* DC. (Rutaceae): IC_{50} = 3–5 µM [21, 22], tiliacorinine [from *Tiliacora triandra* (Colebr.) Diels (Menispermaceae): IC_{50} = 4–7 µM] [23]. Curcumin and analogs (Zingiberaceae): IC_{50} 3–17 µM [24–27], piperlongumine [from *P. longum* L.: IC_{50} = 4–15 µM] [28, 29], luteolin [from *Reseda luteola* L. (Resedaceae): IC_{50} = 10 µM] [30], candidione [from *Derris indica* (Lamk.) Benn.: IC_{50} = 12–17 µM] [31], and plumbagin [from *Plumbago indica* L. (Plumbaginaceae): IC_{50} = 24 µM] [32] showed moderated to potent activities (▶ **Table 1**). Possible molecular mechanisms of these herbs and/or isolated compounds/synthetic analogs on CCA cells involve induction of apoptosis, autophagy, and cell cycle arrest (at G_0/G_1, G_1, G_1/S, or G_2/M phases) through suppression of proinflammatory cytokines and growth factors (IL6, EGF, VEGF, etc.) [10, 13, 23, 27–30, 32, 34, 36, 41, 43, 46, 51–53, 57–85], suppression of expression of cell surface receptors (*Vegfr2*, EGFR peroxisome proliferator-activated receptor gamma, DR4 and DR5, and TRAIL) [69, 86–88], and deregulation of intracellular pathways (JAK/STAT3, RAS/MAPK, PI3K/AKT, GSK*β*/*β*-catenin, NF*κ*B/AMPK, ERK, p38/MAPK, HO1, ROS/JNK, EGFR, VEGF, COX2, FAK, MMP2, MMP9, ICAM1, caspase-3, -8, and − 9, TR1, MDR1, MRP1, 2, and 3, TRAF1, XIAP, p21, p53, p65, and CHOP dependent) (▶ **Fig. 2**) [7, 11, 16, 19, 25, 26, 30, 31, 37, 38, 42, 45, 49, 54, 66, 70, 78, 79, 89–120].

The most advanced development of a potential herb as a chemotherapeutic agent for CCA is *A. lancea* (Thunb.) DC. A series of studies on the research and development of *A. lancea* (Thunb.) DC. was systematically conducted by our research group [121]. *A. lancea* (Thunb.) DC. is a medicinal plant growing in tropical and subtropical zones of East Asia such as China and Japan. Its dried rhizome is commonly used in Chinese ("Cang Zhu"), Japanese campo ("So-jutsu"), and Thai ("Khod-Kha-Mao") traditional medicines for fever, colds, flu, sore throat, rheumatic diseases, digestive disorders, night blindness, influenza, rheumatic diseases, digestive disorders, night blindness, and cancers. Modern pharmacological studies also support the broad pharmacological effects of *A. lancea* (Thunb.) DC. in various diseases [122]. Phytochemical investigations reveal a series of sesquiterpenoids, monoterpenes, polyacetylenes, phenolic acids, and steroids from *A. lancea* (Thunb.) DC. rhizomes [123]. The major constituents are AT (14%), BE (6%), atractylon (2%), and HS (1%). The potential of *A. lancea* (Thunb.) DC. and the two major compounds AT and BE for treatment and control of CCA has extensively been evaluated both *in vitro* (human CCA cell lines) and *in vivo* (xenograft mouse model and OV/DMN-induced CCA hamster model) [121, 124, 125]. Results confirm anti-CCA potential and safety profiles of both the crude *A. lancea* (Thunb.) DC. extract, as well as AT and BE and the finished product [capsule pharmaceutical formulation of the standardized *A. lancea* (Thunb.) DC. extract] [126, 127]. *A. lancea* (Thunb.) DC. and both compounds exhibit potent and selective antiproliferative activities against CCA cells. The IC_{50} values range from 20 to 30 µg/mL, with a selectivity index of 3–5 [128, 129]. The potencies of activity of *A. lancea* (Thunb.) DC. and both compounds on CCA cell growth is about 3- to 4-fold of the standard drug 5-FU. Furthermore, *A. lancea* (Thunb.) DC. extract, AT, and BE inhibit CCA cell invasion and migration and formation of new blood vessels [86, 128, 130–132], suggesting a potential role as an antimetastasis and antiangiogenesis agent for CCA. The potential anticancer and antiangiogenesis properties of *A. lancea* (Thunb.) DC. extract and its major constituents have been demonstrated in various types of cancer, e.g., murine blastoma cells HeLa (human cervical cells), SGC-7901 (human gastric cancer cells), BEL-7402 (human liver cancer cells), H33, S180, HL-60, leukemic cells, and gastric cancer [131–135]. The underlying mechanisms of the antiproliferative effects of *A. lancea* (Thunb.) DC., AT, and BE against CCA cells mainly involve the induction of cell cycle arrest (at G_1 phase) and apoptosis through activation or suppression of molecular targets/signaling pathways involved in CCA pathogenesis. These include the activation of caspase-3/7 and suppression of HO1 production, activation of STAT1/2 and JAK/STAT signaling cascades, suppression of NFκB, and suppression of cytoprotective enzymes and key growth regulatory transcription factors [38, 41, 42, 62, 98–100]. The first-in-human starting dose was estimated from the MRSD (maximum recommended starting dose) from toxicology testing in animals [136], which was 2400 mg for a person weighing 60 kg. Despite the concern of bleeding (antiplatelet aggregation) and adverse effect on the nervous system previously reported *in vitro* and in animals [123], results of phase I clinical trials using 1 g *A. lancea* (Thunb.) DC. (about 50% of the estimated maximum dose in humans) confirmed the safety profile in healthy Thai subjects [124]. The pharmacokinetics of AT was investigated in healthy Thai subjects following a single (1 g) or daily (1 g for 21 days) administration of the capsule formulation of the standardized *A. lancea* (Thunb.) DC. extract [124]. AT was rapidly absorbed but with low systemic bioavailability and a short residence time (within 8 h). The immunostimulatory activity of the standardized *A. lancea* (Thunb.) DC. extract was linked with suppression of the production of TNF-α and IL6 cytokines, which are involved in the pathogenesis and severity of CCA [137]. A phase II dose-finding study is underway to confirm efficacy, tolerability, and immunomodulatory activity of *A. lancea* (Thunb.) DC. in patients with advanced-stage CCA. It is noted for the toxic effect of AT and BE on zebrafish embryo development [86]. Although the results may imply similar toxicity in humans, considering the much more sensitivity of the zebrafish model compared with mammalian cells and rodent models, high intensity of the effect would not be expected in humans. Further studies are needed to confirm this finding.

Apart from *A. lancea* (Thunb.) DC., *C. longa* L., *G. hanburyi* Hook.f., *A. annua* L., *Z. officinale* Roscoe, and *A. paniculata* (Burm. f.) Nees are among the herbs that have been of research interest for anti-CCA development. Curcumin is a major component of *C. longa* or turmeric. It is a dietary constituent with tumor-suppressing potential by inhibiting multiple molecular targets/signaling pathways involved in carcinogenesis, including CCA. Curcumin and synthetic analogs exhibit potent antiprolifertive activities against human CCA cells with IC_{50} values of 3–17 µM [24, 27]. However, clinical uses of curcumin in CCA and other types of cancer may be limited due to its low systemic bioavailability [138]. It inhibits cell migration and induces cell cycle arrest at the G_2/M phase [66]. The action of curcumin in CCA involves multiple molecular targets/signaling pathways, including transcription factors (NFκB, STAT3, and AP1.16), peroxisome proliferator-activated receptor, AKT activation pathway, B-cell lymphoma 2, B-cell lymphoma-extra large, cell survival proteins (cIAP1, cIAP2, and surviving 15), and Notch1 signaling [25, 26, 66, 83, 106, 107, 109, 110].

The anticancer potentials of *G. hanburyi* Hook.f. extract and isolated compounds/synthetic analogs have been well demonstrated in various types of cancer [139]. *G. hanburyi* Hook.f. and its isolated caged xanthones (gambogic acid, forbesione, isomorellin, and isomorellinol, etc.) from the resin and fruits have been used widely in Thai traditional medicine [51]. Gambogic acid was shown to have a favorable safety profile in a phase II a trial in patients with advanced malignant tumors, i.e., lung, gastrointestinal, liver, breast, and renal adenocarcinoma [140]. Neverthess, no clinical study was conducted in patients with advanced-stage CCA. The antiproliferative activity of both the extract (IC_{50} = 2–3 µg/mL) and isolated compounds/synthetic analogs (IC_{50} = 0.03–3 µM) is considered potent [6, 51]. The extract and caged xanthones induce apoptosis via the mitochondrial pathway [51] and induction of G_0/G_1-phase cell cycle arrest through p53 and NFκB signaling pathways [52]. Combinations of isomorellin or forbesione with doxorubicin exhibited a significant synergistic effect on CCA cells through suppression of MRP1, activation of NFκB, enhancement of Bcl2-like protein 4 (Bax)/Bcl2, activation of caspase-9 and caspase-3, and suppression of the expression of survivin, procaspase-9, and procaspase-3 [112]. The combination of forbesione with 5-FU strongly suppressed the expression of

Bcl2 and procaspase-3 while enhancing the expression of p53, Bax, Apaf-1, caspase-9 and caspase-3 compared with single-drug treatment [111]. The safety profile of gambogic acid in humans together with its potent antiproliferative activity against CCA make this compound a strong candidate for further development as a CCA chemotherapeutic agent. In addition, gambonic acid is available in the parenteral formulation, which is suitable for CCA patients.

The sesquiterpene lactones artemisinin and derivatives (artemether, artesunate, arteether, and dihydroartemisinin) derived from *A. annua* L. constitute a unique class of antimalarial drugs with significant potential for drug repurposing for a wide range of diseases, including cancer [141]. The antiproliferative activities of artemisinins against CCA cells are relatively weak (IC_{50} = 75–377 µM) [37]. The mechanisms of their action against CCA have been reported to involve multiple critical biological targets/signaling pathways of CCA pathogenesis, i.e., DAPK1, BECLIN1, Bcl2, PI3KC3, and MCL-1 [61, 96, 97]. The anti-CCA activities have been shown to be through induction of both apoptosis and autophagy-dependent caspase-independent cell death and cell cycle arrest at phases S, G_0/G_1, and G_2/M.

Z. officinale Roscoe, or ginger, is a popular spice used globally, especially in most Asian countries. It has been used as a pain relief for arthritis, muscle soreness, chest pain, low back pain, stomach pain, and menstrual pain. The rhizomes contain over 400 different compounds. The phenolic compounds gingerol and shogaol are found in higher quantities than others. Evidence from *in vitro*, animal, and epidemiological studies suggest that ginger and its active constituents suppress the growth and induce apoptosis of a variety of cancer types, including skin, ovarian, colon, breast, cervical, oral, renal, prostate, gastric, pancreatic, liver, and brain cancer. The active ingredients of ginger, mainly, 6-gingerol and 6-shogaol, target several cellular molecules that contribute to tumorigenesis, cell survival, cell proliferation, invasion, and angiogenesis (NFκB, STAT3, Rb, MAPK, PI3k/Akt Ca^{2+} signals, Akt, ERK, cIAP1, cyclin A, cyclin D1, Cdk, cathepsin D, caspase-3/7, survivin, cIAP1, XIAP, Bcl2, MMP9, ER stress, and eIF2α) [142]. *In vitro* studies showed that ginger has promising antiprolifertive and antioxidant activities against human CCA cells by inducing programmed cell death [11, 84]. The ethanolic extract of ginger exhibits significant tumor growth inhibition, prolongs survival time, and increases survival rate in CCA-xenografted mice and OV/DMN-induced CCA in hamsters. In the xenograft model, the crude extract of ginger produced significant anti-CCA activity compared with cisplatin and the untreated control. The extract at medium (1 g/kg body weight) and high (2 g/kg body weight) dose levels (oral daily dose for 30 days) significantly inhibited tumor growth to about 55.6 and 51.1 % of the untreated control, respectively, while cisplatin inhibited tumor growth to 60 % of the control [84]. Interestingly, significant reduction of lung metastasis was observed in the xenografted mice treated with the crude extract of ginger and cisplatin compared with the untreated control. In OV/DMN-induced CCA hamsters, promising anti-CCA activity of the crude extract of ginger was observed at all dose levels, particularly at the highest oral dose level of 5 g/kg body weight for 30 days [143]. The median survival rate and survival time were significantly prolonged (about two times) in hamsters treated with the extract at all dose levels compared with 5-FU-treated and untreated control groups during the 4–6 months observation period. At week 36, all hamsters except those treated with the highest ginger dose died (1 hamster died, 80 % survival rate). The untreated control animals started to die as early as 14 weeks.

A. paniculata (Burm.f.) Nees is an important herbal medicine widely used in several Asian countries, including China, India, and Thailand, for the treatment of respiratory infection, inflammation, immunostimulation, hepatoprotective, cardioprotective, cold, fever, bacterial dysentery, diarrhea, and hypoglycemic and anticancer activities [144–149]. Recently, the Ministry of Public Health of Thailand has approved *A. paniculata* (Burm.f.) Nees for the treatment of COVID-19 [150]. *A. paniculata* (Burm.f.) Nees and its active compound andrographolide have been shown to inhibit cancer cell migration and invasion, including CCA. Due to their low potencies of activity and requirement of a large dose [151], a number of andrographolide analogs, particularly C19 triphenylmethyl ether substitution (AG050) and its nanoencapsulated formulation, have recently been developed with improved activities against CCA (IC_{50} = 3 µM) [17, 18]. These analogs and nanoformulation exhibit potent activity against CCA cells. The inhibitory effect on CCA cell proliferation is through induction of apoptosis and cell cycle arrest at the G_o/G_1 and G_2/M phases through downregulation of cyclin D1, Bcl2, and caspase-3, while the upregulation of proapoptotic protein Bax and cleavage of poly (ADP-ribose) polymerase occurs [60]. Andrographolide was also shown to inhibit CCA cell invasion and migration via suppression of claudin 1 through the activation of p38 MAPK signaling [19]. The long history of use and relatively safe profile [152] together with evidence of the potency of antiproliferative activity against human CCA cells make *A. paniculata* (Burm.f.) Nees extract or andrographolide a candidate as a repurposed drug for CCA.

Resveratrol and capsaicin are among other reported compounds derived from several plant species that have been investigated for anti-CCA activities [73, 75–77]. Resveratrol is a polyphenol found naturally in red wine, grapes, mulberries, cranberries, and peanuts. The compound exhibits cancer chemopreventive activity through inhibition of tumor initiation, promotion, and progression. In CCA cell lines, resveratrol was shown to interfere with cell cycle progression, resulting in arresting different phases of the cell cycle (G_o/G_1, S, and G_2 phases) to induce apoptosis via the mitochondrial-dependent pathway (caspase-dependent and -independent) [75], to stimulate autophagy, and to suppress IL6 by CAFs secretory product [76]. It also produces the chemosensitizing effect of 5-FU on CCA growth inhibition [73]. Capsaicin, found in hot red chilli peppers [*Capsicum* spp. (Solanaceae)], possesses several pharmacological activities, i.e., analgesic, anti-inflammation, and antiproliferative effects, on different gastrointestinal cancer cells [154]. The anti-CCA activity of capsaicin was shown to be associated with the induction of apoptosis and attenuation of the GLI1 and GLI2 targets of the Hedgehog signaling pathway (role in carcinogenesis) [101–102]. The use of capsaicin as a food supplement to inhibit Hedgehog signaling might therefore be of additional therapeutic benefit in patients with CCA. In the xenograft mouse model, a combination of capsaicin with 5-FU was synergistic and significantly suppressed tumor growth compared with 5-FU alone. Further investigation revealed that

the autophagy induced by 5-FU was inhibited by capsaicin. The mechanism of action was shown to be through the inhibition of 5-FU-induced autophagy by activating the PI3K/AKT/mTOR signaling pathway [103].

Herbs constitute a promising source of medicine for CCA control. The anti-CCA potential of several herbs and isolated compound/synthetic analogs have been demonstrated in different experimental models in conjunction with their underlying mechanisms of action at the molecular and cellular levels. As herbal medicines usually contain several pharmacologically active compounds, their multi-ingredient characteristics may make the evaluation of clinically useful products more complex than synthetic drugs. With regard to the therapeutic aspect, however, using the whole herbal extract would be expected to provide more therapeutic benefit compared to synthetic drugs concerning efficacy (synergistic action) and tolerability (buffering effect). The limitation of the current study includes only articles published in English were included in the analysis and the number of the reported articles may therefore be underestimated. Comparison of the potencies of antiproliferative activities of the investigated plants/isolated or synthetic compounds/herbal formulations was made based on only available data on the IC_{50} values, which were not reported in some studies. Some reported the antiproliferative activity potencies as the percentage of inhibitory effects on cell growth at specified concentrations. In addition, different CCA cell lines and assay methods for assessment of antiproliferative activities were used in different studies.

In conclusion, a number of plants, isolated compounds, synthetic analogs, and herbal formulations have been demonstrated for their potential to control CCA. However, only *A. lancea* (Thunb.) DC. was fully developed based on the reverse pharmacology approach. Future research should be geared toward the full development of the candidate herbs until delivery of final products that are safe and effective for CCA control. Other targets of their action should be further investigated. Research targeting inflammatory, proliferative, and angiogenesis processes, development, and progression has been an extensive area. Blocking the generation of an inflammatory infiltrate by interfering with critical molecules of the adhesion process is an attractive strategy to control CCA.

Materials and Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [156].

Database and search strategy

The literature search was conducted from three databases, i.e., PubMed, ScienceDirect, and Scopus in March-June 2021. The search terms applied were “Cholangiocarcinoma” AND “Herbs” AND/OR “Herbal medicine” AND/OR “Traditional medicine” AND/OR “Plants”. All articles were retrieved and downloaded to the EndNote X9 database (Thomson Reuters Company) for further analysis.

Study selection

Study selection was performed independently by two reviewers. The studies were initially screened by titles and abstracts to exclude irrelevant articles and duplication. Full-text articles included after the screening were further evaluated by applying the predefined eligibility criteria. Studies were eligible if they met the following criteria: (i) published up to May 2021; (ii) available as full text in English; and (iii) with *in vitro*/*in vivo*/clinical studies related to the investigation of the anti-CCA activity of herbal or traditional medicine. The articles were excluded if: (i) there was unclear methodology or insufficient information or (ii) if they were review articles, letters to the editor, editorials, a systematic analysis, or a meta-analysis.

Data extraction

Two reviewers extracted data independently and resolved the disparity by discussion and suggestion from the third reviewer. The following information was extracted: first author’s name and year of publication, name of herbs/herbal extract/herbal medicine or isolated/synthetic analog(s), type of study (*in vitro*/*in vivo*/clinical), objective(s) of the study [investigation of antiproliferative activity alone or with antimetastasis or antiangiogenesis or antioxidative, anti-CCA activity, and mechanism/target(s) of action], and key findings.

Supporting information

Mechanisms of antiproliferative activities in animals are available in the Supporting Information.

Contributors’ Statement

Data collection and analysis: K. Na-Bangchang, T. Plengsuriyakarn; design of the study: K. Na-Bangchang, J. Karbwang; drafting the manuscript: K. Na-Bangchang; critical revision of the manuscript: K. Na-Bangchang, T. Plengsuriyakarn, J. Karbwang.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Banales JM, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, Cardinale V, Carpino G, Andersen JB, Braconi C, Calvisi DF, Perugorria MJ, Fabris L, Boulter L, Macias RIR, Gaudio E, Alvaro D, Gradilone SA, Strazzabosco M, Marzioni M, Coulouarn C, Fouassier L, Raggi C, Invernizzi P, Mertens JC, Moncsek A, Rizvi S, Heimbach J, Koerkamp BG, Bruix J, Forner A, Bridgewater J, Valle JW, Gores GJ. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol 2020; 17: 557–588

[2] Sripa B, Pairojkul C. Cholangiocarcinoma: lessons from Thailand. Curr Opin Gastroenterol 2008; 24: 349–356

[3] Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J; ABC-02 Trial Investigators. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med 2010; 362: 1273–1281

[4] Haque MU, Ferdiousi N, Sajon SR. Anti-cancer agents derived from plant and dietary sources: a review. Int J Pharmacognosy 2016; 3: 55–66

[5] Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A, Na-Bangchang K. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells *in vitro*. BMC Complement Altern Med 2010; 10: 55

[6] Boonyanugomol W, Hahnvajanawong C, Reutrakul V, Anantachok N. Growth Inhibitory Activity of *Garcinia Hanburyi* extracts on Cholangiocarcinoma Cell Lines. Srinagarind Med J 2007; 22: 278–282

[7] Tedasen A, Khoka A, Madla S, Sriwiriyajan S, Graidist P. Anticancer effects of piperine-free *Piper nigrum* extract on cholangiocarcinoma cell lines. Pharmacogn Mag 2020; 16: 28–38

[8] Thongdeeying P, Itharat A, Umehara K, Ruangnoo S. A novel steroid and cytotoxic constituents from *Dioscorea membranacea* Pierre against hepatocellular carcinoma and cholangiocarcinoma cells. J Ethnopharmacol 2016; 194: 91–97

[9] Amuamuta A, Plengsuriyakarn T, Na-Bangchang K. Anticholangiocarcinoma activity and toxicity of the *Kaempferia galanga* Linn. Rhizome ethanolic extract. BMC Complement Altern Med 2017; 17: 213

[10] Tritripmongkol P, Plengsuriyakarn T, Tarasuk M, Na-Bangchang K. *In vitro* cytotoxic and toxicological activities of ethanolic extract of *Kaempferia galanga* Linn. and its active component, ethyl-*p*-methoxycinnamate, against cholangiocarcinoma. J Integr Med 2020; 18: 326–333

[11] Plengsuriyakarn T, Viyanant V, Eursitthichai V, Tesana S, Chaijaroenkul W, Itharat A, Na-Bangchang K. Cytotoxicity, toxicity, and anticancer activity of *Zingiber officinale* Roscoe against cholangiocarcinoma. Asian Pac J Cancer Prev 2012; 13: 4597–4606

[12] Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momosaki S, Kojiro M. The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res 1994; 54: 448–454

[13] Obchoei S, Wongkham S, Aroonkesorn A, Suebsakwong P, Suksamrarn A. Anti-cancer effect of cucurbitacin B on cholangiocarcinoma cells. The 6th International Conference on Biochemistry and Molecular Biology (BMB2018), Ranong Province, Thailand; 2018: 1–7

[14] Tengchaisri T, Chawengkirttikul R, Rachaphaew N, Reutrakul V, Sangsuwan R, Sirisinha S. Antitumor activity of triptolide against cholangiocarcinoma growth *in vitro* and in hamsters. Cancer Lett 1998; 133: 169–175

[15] Ding X, Zhang B, Pei Q, Pan J, Huang S, Yang Y, Zhu Z, Lv Y, Zou X. Triptolide induces apoptotic cell death of human cholangiocarcinoma cells through inhibition of myeloid cell leukemia-1. BMC Cancer 2014; 14: 271

[16] Boueroy P, Saensa-Ard S, Siripong P, Kanthawong S, Hahnvajanawong C. Rhinacanthin-C Extracted from *Rhinacanthus nasutus* (L.) Inhibits Cholangiocarcinoma Cell Migration and Invasion by Decreasing MMP-2, uPA, FAK and MAPK Pathways. Asian Pac J Cancer Prev 2018; 19: 3605–3613

[17] Puntawee S, Theerasilp M, Reabroi S, Saeeng R, Piyachaturawat P, Chairoungdua A, Nasongkla N. Solubility enhancement and *in vitro* evaluation of PEG-b-PLA micelles as nanocarrier of semi-synthetic andrographolide analogue for cholangiocarcinoma chemotherapy. Pharm Dev Technol 2016; 21: 437–444

[18] Sombut S, Bunthawong R, Sirion U, Kasemsuk T, Piyachaturawat P, Suksen K, Suksamrarn A, Saeeng R. Synthesis of 14-deoxy-11, 12-didehydroandrographolide analogues as potential cytotoxic agents for cholangiocarcinoma. Bioorg Med Chem Lett 2017; 27: 5139–5143

[19] Pearngam P, Kumkate S, Okada S, Janvilisri T. Andrographolide inhibits cholangiocarcinoma cell migration by down-regulation of claudin-1 *via* the p-38 signaling pathway. Front Pharmacol 2019; 10: 827

[20] Ma Q, Feng Y, Deng K, Shao H, Sui T, Zhang X, Sun X, Jin L, Ma Z, Luo G. Unique responses of hepatocellular carcinoma and cholangiocarcinoma cell lines toward cantharidin and norcantharidin. J Cancer 2018; 9: 2183–2190

[21] Sribuhom T, Boueroy P, Hahnvajanawong C, Phatchana R, Yenjai C. Benzoyltyramine alkaloids atalantums A–G from the peels of *Atalantia monophylla* and their cytotoxicity against cholangiocarcinoma cell lines. J Nat Prod 2017; 80: 403–408

[22] Sombatsri A, Thummanant Y, Sribuhom T, Boonmak J, Youngme S, Phusrisom S, Kukongviriyapan V, Yenjai C. New limonophyllines A–C from the stem of *Atalantia monophylla* and cytotoxicity against cholangiocarcinoma and HepG2 cell lines. Arch Pharm Res 2018; 41: 431–437

[23] Janeklang S, Nakaew A, Vaeteewoottacharn K, Seubwai W, Boonsiri P, Kismali G, Suksamrarn A, Okada S, Wongkham S. *In vitro* and *in vivo* antitumor activity of tiliacorinine in human cholangiocarcinoma. Asian Pac J Cancer Prev 2014; 15: 7473–7478

[24] Suphim B, Buranrat B, Prawan A, Kukongviriyapan V. Sensitivity of cholangiocarcinoma cells to chemotherapeuticagents and curcumin. Srinagarind Med J 2008; 23: 284–289

[25] Suphim B, Prawan A, Kukongviriyapan U, Kongpetch S, Buranrat B, Kukongviriyapan V. Redox modulation and human bile duct cancer inhibition by curcumin. Food Chem Toxicol 2010; 48: 2265–2272

[26] Yin S, Sokolowski K, Kunnimalaiyaan S, Gamblin TC, Kunnimalaiyaan M. Curcumin-mediated regulation of Notch1/hairy and enhancer of split-1/survivin: molecular targeting in cholangiocarcinoma. J Surg Res 2015; 198: 434–440

[27] Qiu C, Hu Y, Wu K, Yang K, Wang N, Ma Y, Zhu H, Zhang Y, Zhou Y, Chen C, Li S, Fu L, Zhang X, Liu Z. Synthesis and biological evaluation of allylated mono-carbonyl analogues of curcumin (MACs) as anti-cancer agents for cholangiocarcinoma. Bioorg Med Chem Lett 2016; 26: 5971–5976

[28] Thongsom S, Suginta W, Lee KJ, Choe H, Talabnin C. Piperlongumine induces G2/M phase arrest and apoptosis in cholangiocarcinoma cells through the ROS-JNK-ERK signaling pathway. Apoptosis 2017; 22: 1473–1484

[29] Chen SY, Huang HY, Lin HP, Fang CY. Piperlongumine induces autophagy in biliary cancer cells via reactive oxygen species-activated Erk signaling pathway. Int J Mol Med 2019; 44: 1687–1696

[30] Aneknan P, Kukongviriyapan V, Prawan A, Kongpetch S, Sripa B, Senggunprai L. Luteolin arrests cell cycling, induces apoptosis and inhibits the JAK/STAT3 pathway in human cholangiocarcinoma cells. Asian Pac J Cancer Prev 2014; 15: 5071–5076

[31] Kurasug B, Kukongviriyapan V, Prawan A, Yenjai C, Kongpetch S. Antitumor effects of candidone extracted from *Derris indica* (Lamk) Bennet in cholangiocarcinoma cells. Trop J Pharm Res 2018; 17: 1338

[32] Panrit L, Plengsuriyakarn T, Martviset P, Na-Bangchang K. Inhibitory activities of plumbagin on cell migration and invasion and inducing activity on cholangiocarcinoma cell apoptosis. Asian Pac J Trop Med 2018; 11: 430–435

[33] Promraksa B, Phetcharaburanin J, Namwat N, Techasen A, Boonsiri P, Loilome W. Evaluation of anticancer potential of Thai medicinal herb extracts against cholangiocarcinoma cell lines. PLoS One 2019; 14: e0216721

[34] Shen DY, Kang JH, Song W, Zhang WQ, Li WG, Zhao Y, Chen QX. Apoptosis of human cholangiocarcinoma cell lines induced by β-escin through mitochondrial caspase-dependent pathway. Phytother Res 2011; 25: 1519–1526

[35] Intuyod K, Priprem A, Pairojkul C, Hahnvajanawong C, Vaeteewoottacharn K, Pinlaor P, Pinlaor S. Anthocyanin complex exerts anti-cholangiocarcinoma activities and improves the efficacy of drug treatment in a gemcitabine-resistant cell line. Int J Oncol 2018; 52: 1715–1726

[36] Saenglee S, Senawong G, Jogloy S, Sripa B, Senawong T. Peanut testa extracts possessing histone deacetylase inhibitory activity induce apopto-

sis in cholangiocarcinoma cells. Biomed Pharmacother 2018; 98: 233–241

[37] Chaijaroenkul W, Viyanant V, Mahavorasirikul W, Na-Bangchang K. Cytotoxic activity of artemisinin derivatives against cholangiocarcinoma (CL-6) and hepatocarcinoma (Hep-G2) cell lines. Asian Pac J Cancer Prev 2011; 12: 55–59

[38] Mathema VB, Chaijaroenkul W, Na-Bangchang K. Cytotoxic activity and molecular targets of atractylodin in cholangiocarcinoma cells. J Pharm Pharmacol 2019; 71: 185–195

[39] Muhamad N, Plengsuriyakarn T, Chittasupho C, Na-Bangchang K. The potential of atractylodin-loaded PLGA nanoparticles as chemotherapeutic for cholangiocarcinoma. Asian Pac J Cancer Prev 2020; 21: 935–941

[40] Omar AI, Plengsuriyakarn T, Chittasupho C, Na-Bangchang K. Enhanced oral bioavailability and biodistribution of atractylodin encapsulated in PLGA nanoparticle in cholangiocarcinoma. Clin Exp Pharmacol Physiol 2020; 48: 318–328

[41] Kotawong K, Chaijaroenkul W, Muhamad P, Na-Bangchang K. Cytotoxic activities and effects of atractylodin and β-eudesmol on the cell cycle arrest and apoptosis on cholangiocarcinoma cell line. J Pharmacol Sci 2018; 136: 51–56

[42] Mathema VB, Chaijaroenkul W, Karbwang J, Na-Bangchang K. Growth inhibitory effect of β-eudesmol on cholangiocarcinoma cells and its potential suppressive effect on heme oxygenase-1 production, STAT1/3 activation, and NF-κB downregulation. Clin Exp Pharmacol Physiol 2017; 44: 1145–1154

[43] Rattanata N, Klaynongsruang S, Daduang S, Tavichakorntrakool R, Limpaiboon T, Lekphrom R, Boonsiri P, Daduang J. Inhibitory Effects of Gallic Acid Isolated from *Caesalpinia mimosoides* Lam. on Cholangiocarcinoma Cell Lines and Foodborne Pathogenic Bacteria. Asian Pac J Cancer Prev 2016; 17: 1341–1345

[44] Songsiang U, Thongthoom T, Zeekpudsa P, Kukongviriyapan V, Boonyarat C, Wangboonskul J, Yenjai C. Antioxidant activity and cytotoxicity against cholangiocarcinoma of carbazoles and coumarins from *Clausena harmandiana*. Sci. Asia 2012; 38: 75–81

[45] Gu Y, Xiao L, Ming Y, Zheng Z, Li W. Corilagin suppresses cholangiocarcinoma progression through Notch signaling pathway *in vitro* and *in vivo*. Int J Oncol 2016; 48: 1868–1876

[46] Senggunprai L, Thammaniwit W, Kukongviriyapan V, Prawan A, Kaewseejan N, Siriamornpun S. *Cratoxylum formosum* extracts inhibit growth and metastasis of cholangiocarcinoma cells by modulating the NF-κB and STAT3 pathways. Nutr Cancer 2016; 68: 328–341

[47] Saraphon C, Boonloh K, Kukongviriyapan V, Yenjai C. Cytotoxic flavonoids from the fruits of *Derris indica*. J Asian Nat Prod Res 2017; 19: 1198–1203

[48] Decharchoochart P, Suthiwong J, Samatiwat P, Kukongviriyapan V, Yenjai C. Cytotoxicity of compounds from the fruits of *Derris indica* against cholangiocarcinoma and HepG2 cell lines. J Nat Med 2014; 68: 730–736

[49] Svasti J, Srisomsap C, Subhasitanont P, Keeratichamroen S, Chokchaichamnankit D, Ngiwsara L, Chimnoi N, Pisutjaroenpong S, Techasakul S, Chen ST. Proteomic profiling of cholangiocarcinoma cell line treated with pomiferin *from Derris malaccensis*. Proteomics 2005; 5: 4504–4509

[50] Chokchaichamnankit D, Kongjinda V, Khunnawutmanotham N, Chimnoi N, Pisutcharoenpong S, Techasakul S. Prenylated flavonoids from the leaves of *Derris malaccensis* and their cytotoxicity. Nat Prod Commun 2011; 6: 1103–1106

[51] Hahnvajanawong C, Boonyanugomol W, Nasomyon T, Loilome W, Namwat N, Anantachoke N, Tassaneeyakul W, Sripa B, Namwat W, Reutrakul V. Apoptotic activity of caged xanthones from *Garcinia hanburyi* in cholangiocarcinoma cell lines. World J Gastroenterol 2010; 16: 2235–2243

[52] Hahnvajanawong C, Ketnimit S, Pattanapanyasat K, Anantachoke N, Sripa B, Pinmai K, Seubwai W, Reutrakul V. Involvement of p53 and nuclear factor-kappaB signaling pathway for the induction of G1-phase cell cycle arrest of cholangiocarcinoma cell lines by isomorellin. Biol Pharm Bull 2012; 35: 1914–1925

[53] Assawasuparerk K, Vanichviriyakit R, Chotwiwatthanakun C, Nobsathian S, Rawangchue T, Wittayachumnankul B. Scabraside D Extracted from *Holothuria scabra* Induces Apoptosis and Inhibits Growth of Human Cholangiocarcinoma Xenografts in Mice. Asian Pac J Cancer Prev 2016; 17: 511–517

[54] Leardkamolkarn V, Tiamyuyen S, Sripanidkulchai BO. Pharmacological activity of *Kaempferia parviflora* extract against human bile duct cancer cell lines. Asian Pac J Cancer Prev 2009; 10: 695–698

[55] Jaidee R, Kongpetch S, Prawan A, Senggunprai L. Quercetin enhances phenformin in inhibition of cholangiocarcinoma cell growth. Srinagarind Med J 2020; 35: 249–254

[56] Hemtasin C, Kanokmedhakul S, Kanokmedhakul K, Hahnvajanawong C, Soytong K, Prabpai S, Kongsaeree P. Cytotoxic pentacyclic and tetracyclic aromatic sesquiterpenes from *Phomopsis archeri*. J Nat Prod 2011; 74: 609–613

[57] Li Y, Li D, Chen J, Wang S. A polysaccharide from *Pinellia ternata* inhibits cell proliferation and metastasis in human cholangiocarcinoma cells by targeting of Cdc42 and 67 kDa Laminin Receptor (LR). Int J Biol Macromol 2016; 93: 520–525

[58] Rahman HS. Phytochemical analysis and antioxidant and anticancer activities of mastic gum resin from *Pistacia atlantica* subspecies kurdica. Onco Targets Ther 2018; 11: 4559–4572

[59] Kim JH, Liu L, Lee SO, Kim YT, You KR, Kim DG. Susceptibility of cholangiocarcinoma cells to parthenolide-induced apoptosis. Cancer Res 2005; 65: 6312–6320

[60] Suriyo T, Pholphana N, Rangkadilok N, Thiantanawat A, Watcharasit P, Satayavivad J. *Andrographis paniculata* extracts and major constituent diterpenoids inhibit growth of intrahepatic cholangiocarcinoma cells by inducing cell cycle arrest and apoptosis. Planta Med 2014; 80: 533–543

[61] Hu H, Tan C, Liu X, Luo F, Li K. Upregulation of the MCL-1S protein variant following dihydroartemisinin treatment induces apoptosis in cholangiocarcinoma cells. Oncol Lett 2015; 10: 3545–3550

[62] Kotawong K, Chaijaroenkul W, Roytrakul S, Phaonakrop N, Na-Bangchang K. Screening of molecular targets of action of atractylodin in cholangiocarcinoma by applying proteomic and metabolomic approaches. Metabolites 2019; 9: 260

[63] Puthdee N, Vaeteewoottacharn K, Seubwai W, Wonkchalee O, Kaewkong W, Juasook A, Pinlaor S, Pairojkul C, Wongkham C, Okada S, Boonmars T, Wongkham S. Establishment of an allo-transplantable hamster cholangiocarcinoma cell line and its application for *in vivo* screening of anti-cancer drugs. Korean J Parasitol 2013; 51: 711–717

[64] He W, Wang B, Zhuang Y, Shao D, Sun K, Chen J. Berberine inhibits growth and induces G1 arrest and apoptosis in human cholangiocarcinoma QBC939 cells. J Pharmacol Sci 2012; 119: 341–348

[65] Sakonsinsiri C, Kaewlert W, Armartmuntree N, Thanan R, Pakdeechote P. Anti-cancer activity of asiatic acid against human cholangiocarcinoma cells through inhibition of proliferation and induction of apoptosis. Cell Mol Biol (Noisy-le-grand) 2018; 64: 28–33

[66] Yin DL, Liang YJ, Zheng TS, Song RP, Wang JB, Sun BS, Pan SH, Qu LD, Liu JR, Jiang HC, Liu LX. EF24 inhibits tumor growth and metastasis via suppressing NF-kappaB dependent pathways in human cholangiocarcinoma. Sci Rep 2016; 6: 32167

[67] Zhang A, He W, Shi H, Huang X, Ji G. Natural compound oblongifolin C inhibits autophagic flux, and induces apoptosis and mitochondrial dysfunction in human cholangiocarcinoma QBC939 cells. Mol Med Rep 2016; 14: 3179–3183

[68] Zou Y, Li R, Kuang D, Zuo M, Li W, Tong W, Jiang L, Zhou M, Chen Y, Gong W, Liu L, Tou F. Galangin inhibits cholangiocarcinoma cell growth and metastasis through downregulation of microRNA-21 expression. Biomed Res Int 2020; 2020: 5846938

[69] Tanjak P, Thiantanawat A, Watcharasit P, Satayavivad J. Genistein reduces the activation of AKT and EGFR, and the production of IL6 in cholangiocarcinoma cells involving estrogen and estrogen receptors. Int J Oncol 2008; 53: 177–188

[70] Sae-Lao T, Luplertlop N, Janvilisri T, Tohtong R, Bates DO, Wongprasert K. Sulfated galactans from the red seaweed *Gracilaria fisheri* exerts anti-migration effect on cholangiocarcinoma cells. Phytomedicine 2017; 36: 59–67

[71] Zhang FH, Ren HY, Shen JX, Zhang XY, Ye HM, Shen DY. Magnolol suppresses the proliferation and invasion of cholangiocarcinoma cells via inhibiting the NF-κB signaling pathway. Biomed Pharmacother 2017; 94: 474–480

[72] Kim JH, Liu L, Lee SO, Kim YT, You KR, Kim DG. Susceptibility of cholangiocarcinoma cells to parthenolide-induced apoptosis. Cancer Res 2005; 65: 6312–6320

[73] Roncoroni L, Elli L, Dolfini E, Erba E, Dogliotti E, Terrani C, Doneda L, Grimoldi MG, Bardella MT. Resveratrol inhibits cell growth in a human cholangiocarcinoma cell line. Liver Int 2008; 28: 1426–1436

[74] Frampton GA, Lazcano EA, Li H, Mohamad A, DeMorrow S. Resveratrol enhances the sensitivity of cholangiocarcinoma to chemotherapeutic agents. Lab Invest 2010; 90: 1325–1338

[75] Hahnvajanawong C, Ketnimit S, Boonyanugomol W, Pattanapanyasat K, Chamgramol Y, Sripa B, Namwatf N, Pinmaig K, Tassaneeyakulh W, Reutrakul V. Inhibition of cell cycle progression and apoptotic activity of resveratrol in human intrahepatic cholangiocarcinoma cell lines. Asian Biomedicine 2011; 5: 775–786

[76] Thongchot S, Ferraresi A, Vidoni C, Loilome W, Yongvanit P, Namwat N, Isidoro C. Resveratrol interrupts the pro-invasive communication between cancer associated fibroblasts and cholangiocarcinoma cells. Cancer Lett 2018; 430: 160–171

[77] Roncoroni L, Elli L, Braidotti P, Tosi D, Vaira V, Tacchini L, Lombardo V, Branchi F, Scricciolo A, Doneda L. Transglutaminase 2 mediates the cytotoxicity of resveratrol in a human cholangiocarcinoma and gallbladder cancer cell lines. Nutr Cancer 2018; 70: 761–769

[78] Junking M, Rattanaburee T, Panya A, Budunova I, Haegeman G, Yenchitsomanus PT. Anti-proliferative effects of compound a and its effect in combination with cisplatin in cholangiocarcinoma cells. Asian Pac J Cancer Prev 2020; 21: 2673–2681

[79] Yang X, Wang S, Mu Y, Zheng Y. Schisandrin B inhibits cell proliferation and induces apoptosis in human cholangiocarcinoma cells. Oncol Rep 2016; 36: 1799–1806

[80] Li Y, Ke Y, Zou H, Wang K, Huang S, Rengarajan T, Wang L. Gold nanoparticles synthesized from *Strychni* semen and its anticancer activity in cholangiocarcinoma cell (KMCH-1). Artif Cells Nanomed Biotechnol 2019; 47: 1610–1616

[81] Naus PJ, Henson R, Bleeker G, Wehbe H, Meng F, Patel T. Tannic acid synergizes the cytotoxicity of chemotherapeutic drugs in human cholangiocarcinoma by modulating drug efflux pathways. J Hepatol 2007; 46: 222–229

[82] Lang M, Henson R, Braconi C, Patel T. Epigallocatechin-gallate modulates chemotherapy-induced apoptosis in human cholangiocarcinoma cells. Liver Int 2009; 29: 670–677

[83] Wonkchalee N, Boonmars T, Laummaunwai P, Aromdee C, Hahnvajanawong C, Wu Z, Sriraj P, Aukkanimart R, Chamgramol Y, Pairojkul C, Juasook A, Sudsarn P. A combination of praziquantel and the traditional medicinal plant on *Opisthorchis viverrini* infection and cholangiocarcinoma in a hamster model. Parasitol Res 2013; 112: 4211–4219

[84] Thatte U, Bagadey S, Dahanukar S. Modulation of programmed cell death by medicinal plants. Cell Mol Biol (Noisy-le-grand) 2000; 46: 199–214

[85] Na-Bangchang K, Plengsuriyakarn T, Karbwang J. Research and development of *Atractylodes lancea* (Thunb.) DC. as a promising candidate for cholangiocarcinoma chemotherapeutics. Evid Based Complement Alternat Med 2017; 2017: 5929234

[86] Tshering G, Plengsuriyakarn T, Na-Bangchang K, Pimtong W. Embryotoxicity evaluation of atractylodin and β-eudesmol using the zebrafish model. Comp Biochem Physiol C Toxicol Pharmacol 2021; 239: 108869

[87] Prakobwong S, Gupta SC, Kim JH, Sung B, Pinlaor P, Hiraku Y, Wongkham S, Sripa B, Pinlaor S, Aggarwal BB. Curcumin suppresses proliferation and induces apoptosis in human biliary cancer cells through modulation of multiple cell signaling pathways. Carcinogenesis 2011; 32: 1372–1380

[88] Panichakul T, Intachote P, Wongkajorsilp A, Sripa B, Sirisinha S. Triptolide sensitizes resistant cholangiocarcinoma cells to TRAIL-induced apoptosis. Anticancer Res 2006; 26: 259–265

[89] Leelawat S, Leelawat K. Molecular mechanisms of cholangiocarcinoma cell inhibition by medicinal plants. Oncol Lett 2017; 13: 961–966

[90] Kukongviriyapan V, Phromsopha N, Tassaneeyakul W, Kukongviriyapan U, Sripa B, Hahnvajanawong V, Bhudhisawasdi V. Inhibitory effects of polyphenolic compounds on human arylamine N-acetyltransferase 1 and 2. Xenobiotica 2006; 36: 15–28

[91] Zhao X, Wen F, Wang W, Lu Z, Guo Q. *Actinidia arguta* (Hardy Kiwi) root extract exerts anti-cancer effects via Mcl-1-mediated apoptosis in cholangiocarcinoma. Nutr Cancer 2019; 71: 246–256

[92] Huang GL, Shen DY, Cai CF, Zhang QY, Ren HY, Chen QX. β-escin reverses multidrug resistance through inhibition of the GSK3β/β-catenin pathway in cholangiocarcinoma. World J Gastroenterol 2015; 21: 1148–1157

[93] Xie K, Nian J, Zhu X, Geng X, Liu F. Modulatory role of garlicin in migration and invasion of intrahepatic cholangiocarcinoma via PI3K/AKT pathway. Int J Clin Exp Pathol 2015; 8: 14028–14033

[94] Müller A, Barat S, Chen X, Bui KC, Bozko P, Malek NP, Plentz RR. Comparative study of antitumor effects of bromelain and papain in human cholangiocarcinoma cell lines. Int J Oncol 2016; 48: 2025–2034

[95] Xiao M, Fan X, Fu Y, Zhou Y, Liu S, Peng S. Deoxypodophyllotoxin induces cell cycle arrest and apoptosis in human cholangiocarcinoma cells. Oncol Lett 2018; 16: 3177–3182

[96] Thongchot S, Vidoni C, Ferraresi A, Loilome W, Yongvanit P, Namwat N, Isidoro C. Dihydroartemisinin induces apoptosis and autophagy-dependent cell death in cholangiocarcinoma through a DAPK1-BECLIN1 pathway. Mol Carcinog 2018; 57: 1735–1750

[97] Hu H, Wang Z, Tan C, Liu X, Zhang H, Li K. Dihydroartemisinin/miR-29b combination therapy increases the pro-apoptotic effect of dihydroartemisinin on cholangiocarcinoma cell lines by regulating Mcl-1 expression. Adv Clin Exp Med 2020; 29: 911–919

[98] Acharya B, Chaijaroenkul W, Na-Bangchang K. Atractylodin inhibited the migration and induced autophagy in cholangiocarcinoma cells via PI3K/AKT/mTOR and p38MAPK signalling pathways. J Pharm Pharmacol 2021; 73: 1191–1200. doi:10.1093/jpp/rgab036

[99] Kotawong K, Chaijaroenkul W, Roytrakul S, Phaonakrop N, Na-Bangchang K. Proteomics analysis for identification of potential cell signaling pathways and protein targets of actions of atractylodin and β-eudesmol against cholangiocarcinoma. Asian Pac J Cancer Prev 2020; 21: 621–628

[100] Kotawong K, Chajaroenkul W, Roytrakul S, Phaonakrop N, Na-Bangchang K. The proteomics and metabolomics analysis for screening the molecular targets of action of β-eudesmol in cholangiocarcinoma. Asian Pac J Cancer Prev 2021; 22: 909–918

[101] Wutka A, Palagani V, Barat S, Chen X, El Khatib M, Götze J, Belahmer H, Zender S, Bozko P, Malek NP, Plentz RR. Capsaicin treatment attenuates cholangiocarcinoma carcinogenesis. PLoS One 2014; 9: e95605

[102] Lee GR, Jang SH, Kim CJ, Kim AR, Yoon DJ, Park NH, Han IS. Capsaicin suppresses the migration of cholangiocarcinoma cells by down-regulating matrix metalloproteinase-9 expression via the AMPK-NF-κB signaling pathway. Clin Exp Metastasis 2014; 31: 897–907

[103] Hong ZF, Zhao WX, Yin ZY, Xie CR, Xu YP, Chi XQ, Zhang S, Wang XM. Capsaicin enhances the drug sensitivity of cholangiocarcinoma

through the inhibition of chemotherapeutic-induced autophagy. PLoS One 2015; 10: e0121538

[104] Zhong F, Yang J, Tong ZT, Chen LL, Fan LL, Wang F, Zha XL, Li J. Guggulsterone inhibits human cholangiocarcinoma Sk-ChA-1 and Mz-ChA-1 cell growth by inducing caspase-dependent apoptosis and downregulation of survivin and Bcl-2 expression. Oncol Lett 2015; 10: 1416–1422

[105] Zhong F, Tong ZT, Fan LL, Zha LX, Wang F, Yao MQ, Gu KS, Cao YX. Guggulsterone-induced apoptosis in cholangiocarcinoma cells through ROS/JNK signaling pathway. Am J Cancer Res 2016; 6: 226–237

[106] Khoontawad J, Intuyod K, Rucksaken R, Hongsrichan N, Pairojkul C, Pinlaor P, Boonmars T, Wongkham C, Jones A, Plieskatt J, Potriquet J, Bethony JM, Mulvenna J, Pinlaor S. Discovering proteins for chemoprevention and chemotherapy by curcumin in liver fluke infection-induced bile duct cancer. PLoS One 2018; 13: e0207405

[107] San TT, Khaenam P, Prachayasittikul V, Sripa B, Kunkeaw N, Chan-On W. Curcumin enhances chemotherapeutic effects and suppresses ANGPTL4 in anoikis-resistant cholangiocarcinoma cells. Heliyon 2020; 6: e03255

[108] Prakobwong S, Khoontawad J, Yongvanit P, Pairojkul C, Hiraku Y, Sithithaworn P, Pinlaor P, Aggarwal BB, Pinlaor S. Curcumin decreases cholangiocarcinogenesis in hamsters by suppressing inflammation-mediated molecular events related to multistep carcinogenesis. Int J Cancer 2011; 129: 88–100

[109] Bisht S, Nolting J, Wenzel J, Brossart P, Feldmann G. EF24 suppresses cholangiocellular carcinoma progression, inhibits STAT3 phosphorylation, and induces apoptosis via ROS-mediated oxidative stress. J Oncol 2019; 2019: 8701824

[110] Sato A, Kudo C, Yamakoshi H, Uehara Y, Ohori H, Ishioka C, Iwabuchi Y, Shibata H. Curcumin analog GO-Y030 is a novel inhibitor of IKKβ that suppresses NF-κB signaling and induces apoptosis. Cancer Sci 2011; 102: 1045–1051

[111] Boueroy P, Hahnvajanawong C, Boonmars T, Saensa-Ard S, Wattanawongdon W, Kongsanthia C, Salao K, Wongwajana S, Anantachoke N, Reutrakul V. Synergistic effect of forbesione from *Garcinia hanburyi* in combination with 5-fluorouracil on cholangiocarcinoma. Asian Pac J Cancer Prev 2017; 18: 3343–3351

[112] Hahnvajanawong C, Wattanawongdon W, Chomvarin C, Anantachoke N, Kanthawong S, Sripa B, Reutrakul V. Synergistic effects of isomorellin and forbesione with doxorubicin on apoptosis induction in human cholangiocarcinoma cell lines. Cancer Cell Int 2014; 14: 68

[113] Wang Y, Jiang W, Li C, Xiong X, Guo H, Tian Q, Li X. Autophagy suppression accelerates apoptosis induced by norcantharidin in cholangiocarcinoma. Pathol Oncol Res 2020; 26: 1697–1707

[114] Xu D, Ma Y, Zhao B, Li S, Zhang Y, Pan S, Wu Y, Wang J, Wang D, Pan H, Liu L, Jiang H. Thymoquinone induces G2/M arrest, inactivates PI3K/Akt and nuclear factor-κB pathways in human cholangiocarcinomas both *in vitro* and *in vivo*. Oncol Rep 2014; 31: 2063–2070

[115] Talabnin C, Talabnin K, Wongkham S. Enhancement of piperlongumine chemosensitivity by silencing heme oxygenase-1 expression in cholangiocarcinoma cell lines. Oncol Lett 2020; 20: 2483–2492

[116] Kittiratphatthana N, Kukongviriyapan V, Prawan A, Senggunprai L. Luteolin induces cholangiocarcinoma cell apoptosis through the mitochondrial-dependent pathway mediated by reactive oxygen species. J Pharm Pharmacol 2016; 68: 1184–1192

[117] Zhang J, Su G, Tang Z, Wang L, Fu W, Zhao S, Ba Y, Bai B, Yue P, Lin Y, Bai Z, Hu J, Meng W, Qiao L, Li X, Xie X. Curcumol exerts anticancer effect in cholangiocarcinoma cells via down-regulating CDKL3. Front Physiol 2018; 9: 234

[118] Seubwai W, Vaeteewoottacharn K, Hiyoshi M, Suzu S, Puapairoj A, Wongkham C, Okada S, Wongkham S. Cepharanthine exerts antitumor activity on cholangiocarcinoma by inhibiting NF-kappaB. Cancer Sci 2010; 101: 1590–1595

[119] Uthaisar K, Seubwai W, Srikoon P, Vaeteewoottacharn K, Sawanyawisuth K, Okada S, Wongkham S. Cepharanthine suppresses metastatic potential of human cholangiocarcinoma cell lines. Asian Pac J Cancer Prev 2012; 13: 149–154

[120] Klungsaeng S, Kukongviriyapan V, Prawan A, Kongpetch S, Senggunprai L. Cucurbitacin B induces mitochondrial-mediated apoptosis pathway in cholangiocarcinoma cells *via* suppressing focal adhesion kinase signaling. Naunyn Schmiedebergs Arch Pharmacol 2019; 392: 271–278

[121] Plengsuriyakarn T, Viyanant V, Eursitthichai V, Picha P, Kupradinun P, Itharat A, Na-Bangchang K. Anticancer activities against cholangiocarcinoma, toxicity and pharmacological activities of Thai medicinal plants in animal models. BMC Complement Altern Med 2012; 12: 23

[122] Koonrungsesomboon N, Na-Bangchang K, Karbwang J. Therapeutic potential and pharmacological activities of *Atractylodes lancea* (Thunb.) DC. Asian Pac J Trop Med 2014; 7: 421–428

[123] Jun X, Fu P, Lei Y, Cheng P. Pharmacological effects of medicinal components of *Atractylodes lancea* (Thunb.) DC. Chin Med 2018; 13: 59

[124] Na-Bangchang K, Kulma I, Plengsuriyakarn T, Tharavanij T, Kotawng K, Chemung A, Muhamad N, Karbwang J. Phase I clinical trial to evaluate the safety and pharmacokinetics of capsule formulation of the standardized extract of *Atractylodes lancea*. J Tradit Complement Med 2021; 11: 343–355

[125] Plengsuriyakarn T, Matsuda N, Karbwang J, Viyanant V, Hirayama K, Na-Bangchang K. Anticancer activity of *Atractylodes lancea* (Thunb.) DC in a hamster model and application of PET-CT for early detection and monitoring progression of cholangiocarcinoma. Asian Pac J Cancer Prev 2015; 16: 6279–6284

[126] Rattanathada T, Plengsuriyakarn T, Asasujarit R, Cheoymang A, Karbwang J, Na-Bangchang K. Development of oral pharmaceutical formulation of standardized crude ethanolic extract of *Atractylodes lancea* (Thunb.) DC. JCPS 2020; 29: 280–293

[127] Plengsuriyakarn T, Karbwang J, Na-Bangchang K. Anticancer activity using positron emission tomography-computed tomography and pharmacokinetics of β-eudesmol in human cholangiocarcinoma xenografted nude mouse model. Clin Exp Pharmacol Physiol 2015; 42: 293–304

[128] Plengsuriyakarn T, Viyanant V, Eursitthichai V, Itharat A, Na-Bangchang K. *In vitro* investigations on the potential roles of Thai medicinal plants in treatment of cholangiocarcinoma. Int J Pharm Pharmacol 2012; 2: 1–12

[129] Martviset P, Chaijaroenkul W, Muhamad P, Na-Bangchang K. Bioactive constituents isolated from *Atractylodes lancea* (Thunb.) DC. rhizome exhibit synergistic effect against cholangiocarcinoma cell. J Exp Pharmacol 2018; 10: 59–64

[130] Kimura M, Nojima H, Muroi M, Kimura I. Mechanism of the blocking action of beta-eudesmol on the nicotinic acetylcholine receptor channel in mouse skeletal muscles. Neuropharmacology 1991; 30: 835–841

[131] Tsuneki H, Ma EL, Kobayashi S, Sekizaki N, Maekawa K, Sasaoka T, Wang MW, Kimura I. Antiangiogenic activity of beta-eudesmol *in vitro* and *in vivo*. Eur J Pharmacol 2005; 512: 105–115

[132] Ma EL, Li YC, Tsuneki H, Xiao JF, Xia MY, Wang MW, Kimura I. Beta-eudesmol suppresses tumour growth through inhibition of tumour neovascularisation and tumour cell proliferation. J Asian Nat Prod Res 2008; 10: 159–167

[133] Mazzio EA, Soliman KF. *In vitro* screening of tumoricidal properties of international medicinal herbs: part II. Phytother Res 2010; 24: 1813–1824

[134] Zhao M, Wang Q, Ouyang Z, Han B, Wang W, Wei Y, Wu Y, Yang B. Selective fraction of *Atractylodes lancea* (Thunb.) DC. and its growth inhibitory effect on human gastric cancer cells. Cytotechnology 2014; 66: 201–208

[135] Masuda Y, Kadokura T, Ishii M, Takada K, Kitajima J. Hinesol, a compound isolated from the essential oils of *Atractylodes lancea* rhizome, inhibits cell growth and induces apoptosis in human leukemia HL-60 cells. J Nat Med 2015; 69: 332–339

[136] Contrera JF, Matthews EJ, Kruhlak NL, Benz RD. Estimating the safe starting dose in phase I clinical trials and no observed effect level based on QSAR modeling of the human maximum recommended daily dose. Regul Toxicol Pharmacol 2004; 40: 185–206

[137] Kulma I, Panrit L, Plengsuriyakarn T, Chaijaroenkul W, Warathumpitak S, Na-Bangchang K. A randomized placebo-controlled phase I clinical trial to evaluate the immunomodulatory activities of *Atractylodes lancea* (Thunb.) DC. in healthy Thai subjects. BMC Complement Med Ther 2021; 21: 61

[138] Liu W, Zhai Y, Heng X, Che FY, Chen W, Sun D, Zhai G. Oral bioavailability of curcumin: problems and advancements. J Drug Target 2016; 24: 694–702

[139] Jia B, Li S, Hu X, Zhu G, Chen W. Recent research on bioactive xanthones from natural medicine: *Garcinia hanburyi*. AAPS PharmSciTech 2015; 16: 742–758

[140] Chi Y, Zhan XK, Yu H, Xie GR, Wang ZZ, Xiao W, Wang YG, Xiong FX, Hu JF, Yang L, Cui CX, Wang JW. An open-labeled, randomized, multicenter phase II a study of gambogic acid injection for advanced malignant tumors. Chin Med J (Engl) 2013; 126: 1642–1646

[141] Augustin Y, Staines HM, Krishna S. Artemisinins as a novel anti-cancer therapy: Targeting a global cancer pandemic through drug repurposing. Pharmacol Ther 2020; 216: 107706

[142] Prasad S, Tyagi AK. Ginger and its constituents: role in prevention and treatment of gastrointestinal cancer. Gastroenterol Res Pract 2015; 2015: 142979

[143] Plengsuriyakarn T, Na-Bangchang K. Preclinical toxicology and antichocholangiocarcinoma activity of oral formulation of standardized extract of *Zingiber Officinale*. Planta Med 2021; 86: 104–112

[144] Sareer O, Ahmad S, Umar S. Andrographis paniculata: a critical appraisal of extraction, isolation and quantification of andrographolide and other active constituents. Nat Prod Res 2014; 28: 2081–2101

[145] Gupta S, Mishra KP, Ganju L. Broad-spectrum antiviral properties of andrographolide. Arch Virol 2017; 162: 611–623

[146] Islam MT. Andrographolide, a new hope in the prevention and treatment of metabolic syndrome. Front Pharmacol 2017; 8: 571

[147] Yang SL, Kuo FH, Chen PN, Hsieh YH, Yu NY, Yang WE, Hsieh MJ, Yang SF. Andrographolide suppresses the migratory ability of human glioblastoma multiforme cells by targeting ERK1/2-mediated matrix metalloproteinase-2 expression. Oncotarget 2017; 8: 105860–105872

[148] Lim JC, Chan TK, Ng DS, Sagineedu SR, Stanslas J, Wong WS. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. Clin Exp Pharmacol Physiol 2012; 39: 300–310

[149] Mishra SK, Tripathi S, Shukla A, Oh SH, Kim HM. Andrographolide and analogues in cancer prevention. Front Biosci (Elite Ed) 2015; 7: 255–266

[150] Yearsley C. Thailand approves asian herb andrographis to treat COVID-19. HerbalGram 2021; 129: 35–36

[151] Sa-Ngiamsuntorn K, Suksatu A, Pewkliang Y, Thongsri P, Kanjanasirirat P, Manopwisedjaroen S, Charoensutthivarakul S, Wongtrakoongate P, Pitiporn S, Chaopreecha J, Kongsomros S, Jearawuttanakul K, Wannalo W, Khemawoot P, Chutipongtanate S, Borwornpinyo S, Thitithanyanont A, Hongeng S. Anti-SARS-CoV-2 activity of andrographis paniculata extract and its major component andrographolide in human lung epithelial cells and cytotoxicity evaluation in major organ cell representatives. J Nat Prod 2021; 84: 1261–1270

[152] Worakunphanich W, Thavorncharoensap M, Youngkong S, Thadanipon K, Thakkinstian A. Safety of *Andrographis paniculata*: A systematic review and meta-analysis. Pharmacoepidemiol Drug Saf 2010; 30: 727–739

[153] Hahnvajanawong C, Ketnimit S, Boonyanugomol W, Pattanapanyasat K, Chamgramol Y, Sripa B, Namwatf N, Pinmaig K, Tassaneeyakulh W, Reutrakul V. Inhibition of cell cycle progression and apoptotic activity of resveratrol in human intrahepatic cholangiocarcinoma cell lines. Asian Biomed 2011; 5: 775–786

[154] Friedman JR, Nolan NA, Brown KC, Miles SL, Akers AT, Colclough KW, Seidler JM, Rimoldi JM, Valentovic MA, Dasgupta P. Anticancer activity of natural and synthetic capsaicin analogs. J Pharmacol Exp Ther 2018; 364: 462–473

[155] Saif MW, Lansigan F, Ruta S, Lamb L, Mezes M, Elligers K, Grant N, Jiang ZL, Liu SH, Cheng YC. Phase I study of the botanical formulation PHY906 with capecitabine in advanced pancreatic and other gastrointestinal malignancies. Phytomedicine 2010; 17: 161–169

[156] Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, McKenzie JE. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ 2021; 372: n160