

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

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Key words

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 criteria 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 (IC₅₀ < 15 µg/mL). Cucurbitacin B and triptolide are herbal isolated compounds that exhibit the most promising activities (IC₅₀ < 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 atractyloidin 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

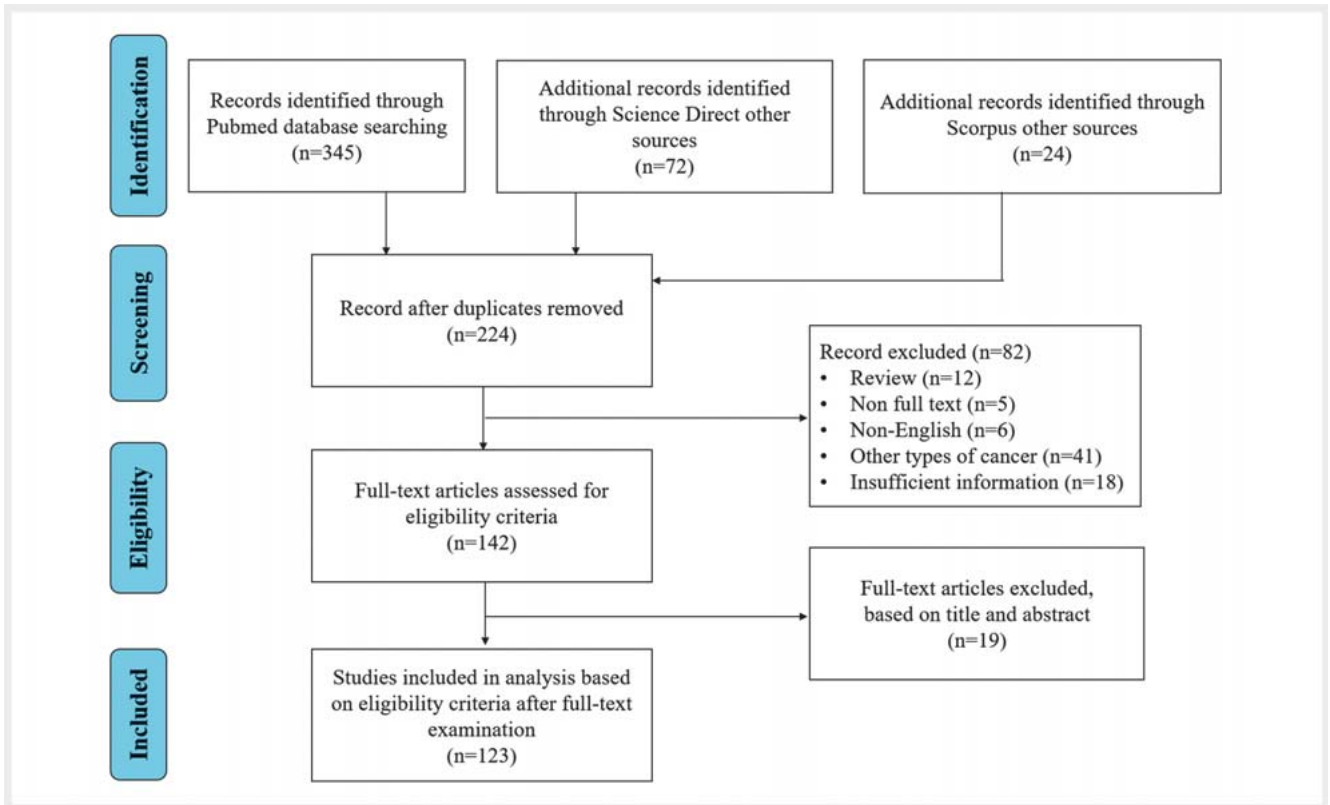
5-FU	5-fluorouracil
ADP	adenosine diphosphate
AKT	protein kinase B
ALNP	atractylodin-loaded PLGA nanoparticle
AMPK	5' AMP-activated protein kinase
AP1	activator protein 1
Apaf-1	apoptotic protease-activating factor 1
AT	atractylodin
Bax	Bcl2-associated X protein
Bcl2	B-cell lymphoma 2
BE	beta-eudesmol
BID	twice per day
CAF	cancer-associated fibroblast
CCA	cholangiocarcinoma
Cdk	cyclin-dependent kinase
CHOP	C/EBP homologues protein
clAP	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
eIF2 α	eukaryotic initiation factor 2
E _{max}	maximum drug effect
EMSA	electrophoresis mobility shift assay
EMPC	ethyl- <i>p</i> -methoxycinnamate
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
GLI1	glioma-associated oncogene homologue 1
GSK β	glycogen synthase kinase beta
HO1	heme oxygenase 1
HS	hinesol
ICAM1	intercellular adhesion molecule 1
IFN γ	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
NF κ B	nuclear factor kappa light chain enhancer of activated B cells
NK	natural killer
OV	<i>Opisthorchis viverrini</i>
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/synthetic compounds (underlined) under investigation and available antiproliferative activity against CCA cell lines.

References	Plants/Active compounds (Family)	Antiproliferative activity
[5]	<u>Crude ethanol extracts:</u> <i>Amomum testaceum</i> Ridl. (Zingiberaceae), <i>Angelica dahurica</i> (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. (Apiaceae), <i>Angelica sinensis</i> (Oliv.) Diels. (Apiaceae), <i>Anethum graveolens</i> L. (Apiaceae), <i>Artemisia annua</i> L. (Compositae), <i>Asclepias curassavica</i> L. (Apocynaceae), <i>Atractylodes lancea</i> (Thunb.) DC. (Compositae), <i>Cuminum cyminum</i> L. (Apiaceae), <i>Curcuma longa</i> L. (Zingiberaceae), <i>Dioscorea membranacea</i> Pierre ex Prain & Burkill (Dioscoreaceae), <i>Dracaena loureirin</i> Gagnep. (Asparagaceae), <i>Foeniculum vulgare</i> Mill. (Apiaceae), <i>Kaempferia galanga</i> L. (Zingiberaceae), <i>Ligusticum sinense</i> Oliv. (Apiaceae), <i>Mammea siamensis</i> Kosterm. (Guttiferae), <i>Mesua ferrea</i> L. (Calophyllaceae), <i>Mimusops elengi</i> L. (Sapotaceae), <i>Myristica fragrans</i> Houtt. (Myristicaceae), <i>Nigella sativa</i> L. (Ranunculaceae), <i>Piper chaba</i> Hunt. (Piperaceae), <i>Piper interruptum</i> Opiz. (Piperaceae), <i>Piper sarmentosum</i> Roxb. (Piperaceae), <i>Plumbago indica</i> L. (Plumbaginaceae), <i>Smilax corbularia</i> Kunth (Smilacaceae), <i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry (Myrtaceae), <i>Zingiber officinale</i> Roscoe (Zingiberaceae), <i>Zingiber ligulatum</i> Roxb. (Zingiberaceae), Ben-ja-Kul 1 formulation, Ben-ja-Kul 2 formulation, Pra-Sa-Práo-Yhai formulation, Tein-5 formulation	<i>A. lancea</i> (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) = <i>K. galanga</i> L. (Zingiberaceae), <i>Z. officinale</i> Roscoe (Zingiberaceae), <i>P. chaba</i> Hunt. (Piperaceae), <i>M. ferrea</i> L. (Calophyllaceae), and Pra-Sa-Práo-Yhai formulation (IC ₅₀ s of 37.36, 34.26, 40.74, 48.23, 44.12 µg/mL, respectively)
[33]	<u><i>Cardiospermum halicacabum</i> L.</u> (Sapindaceae), <u><i>Gomphrena celosioides</i> Mart.</u> (Amaranthaceae), <u><i>Scoparia dulcis</i> L.</u> (Plantaginaceae) (ethanolic extracts)	<i>S. dulcis</i> L. (Plantaginaceae): most potent (56–75% growth inhibition on K KU-100 and K KI-213 cells at 250 µg/mL for 72 h)
[17]	<u><i>Andrographis paniculata</i> (Burm.f.) Nees</u> (Acanthaceae)/ <u>Semisynthetic andrographolide analog (19-triphenylmethyl ether andrographolide, AG 050)</u>	Excellent activity against K KU-M213 cells (IC ₅₀ = 3.33 µM)
[18]	<u><i>Andrographis paniculata</i> (Burm.f.) Nees</u> (Acanthaceae)/ <u>14-deoxy-11,12-didehydroandrographolide analogs</u>	Analogs 5a, 5b: most potent and selective against K KU-M213 cells (IC ₅₀ = 3.37, 3.08 µM); K KU-100 = 2.93, 3.27 µM
[19]	<u><i>Andrographis paniculata</i> (Burm.f.) Nees</u> (Acanthaceae)/ <u>Andrographolide</u>	Significant activity against K KU-100 cells (IC ₅₀ ~ 120 µM)
[34]	<u><i>Aesculus hippocastanum</i> L.</u> (Sapindaceae)/ <u>β-escin</u>	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]	<u>Anthocyanin complex</u> [from cobs of purple way corn Zeamays, certina Kulesh, and petals of blue butterfly pes <i>Clitoria ternatea</i> L. (Leguminosae)]	IC ₅₀ for K KU-213 cells = 620 µg/mL
[36]	<u><i>Arachis hypogaea</i> L.</u> (Leguminosae)/ <u>Peanut testa extract, KK4 and ICG 15042</u>	Potent activity against K KU-M214 cells (KK4: IC ₅₀ = 38.28 µg/mL; ICG15042: IC ₅₀ = 43.91 µg/mL) and K KU-100 cells: (KK4: IC ₅₀ = 78.40 µg/mL; ICG15042: IC ₅₀ = 82.77 µg/mL) at 72 h
[37]	<u><i>Artemisia annua</i> L.</u> (Compositae)/ <u>Artemisinins</u>	Potent activity against CL6 cells: IC ₅₀ = 339 µM (artemisinin), 131 µM (artesanate), 354 µM (β-artemeter), 75 µM (dihydroartemisinin)

continued

► **Table 1** *Continued*

References	Plants/Active compounds (Family)	Antiproliferative activity
[21]	<i>Atalantia monophylla</i> DC. (Rutaceae)/ <u>7 new benzoyltyramines, atalantums A–G (1–7) and 5 known compounds</u>	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]	<i>Atalantia monophylla</i> DC. (Rutaceae)/ <u>limonophyllines A–C (1, 4, 5), limonoids (2, 3), acridone alkaloids (6–16)</u>	Compounds 12, 14, 16: activity against KKU-M156 cells (IC ₅₀ = 3.39–4.1 μg/mL)
[38]	<i>Atractylodes lancea</i> (Thunb.) DC. (Compositae)/ <u>Atractylodin</u>	IC ₅₀ = 216.8 μM for CL6 cells
[39]	<i>Atractylodes lancea</i> (Thunb.) DC. (Compositae)/ <u>Atractylodin (AT) and Atractylodin-loaded PLGA nanoparticle (ALNPs)</u>	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]	<i>Atractylodes lancea</i> (Thunb.) DC. (Compositae)/ <u>Atractylodin (AT). Atractylodin-loaded PLGA nanoparticle (ALNPs)</u>	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]	<i>Atractylodes lancea</i> (Thunb.) DC. (Compositae)/ <u>Atractylodin and β-eudesmol</u>	IC ₅₀ for CL6 cells: atractylodin = 41.66 μg/mL, β-eudesmol = 39.33 μg/mL
[42]	<i>Atractylodes lancea</i> (Thunb.) DC. (Compositae)/ <u>β-eudesmol</u>	IC ₅₀ for CL6 cells = 166 μM
[43]	<i>Caesalpinia mimosoides</i> Lam. (Leguminosae) ethylacetate extract/ <u>Gallic acid (natural: nGA, commercial: cGA)</u>	IC ₅₀ : nGA = 120 μM (M213 cells) and 124 μM (M214 cells cGA), 119 μM (M213 cells), and 147 μM (M214 cells)
[44]	<i>Clausena harmandiana</i> (Pierre) Pierre ex Guillaumin (Rutaceae) hexane, ethyl acetate, methanol extracts/ <u>isolated and purified 12 azarbazoles and coumarins</u>	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]	<u>Corilagin</u> (natural plant polyphenol tannic acid)	IC ₅₀ for QBC9939 and MZ-Cha-1 cells = 39.73 and 36.88 μM, respectively
[46]	<i>Cratoxylum formosum</i> (Jack) Benth. & Hook. f. ex Dyer (Hypericaceae) <u>aqueous and ethanolic Dyer leaf extract</u>	Potent activity (IC ₅₀ for the aqueous extract = 11.3 μg/mL, ethanolic extract = 12.1 μg/mL)
[24]	<i>Curcuma longa</i> L. (Zingiberaceae)/ <u>Curcumin</u>	IC ₅₀ = 5–17 μM (sensitive) for KKU-100, KKU-214, and KKU-OCA17 cells
[25]	<i>Curcuma longa</i> L. (Zingiberaceae)/ <u>Curcumin</u>	IC ₅₀ = 5.9 μM for KKU-214 cells
[26]	<i>Curcuma longa</i> L. (Zingiberaceae)/ <u>Curcumin</u>	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, respectively
[27]	<i>Curcuma longa</i> L. (Zingiberaceae) <u>New allylated mono-carbonyl curcumin analogs (MACs)</u>	Compound 6c: potent activity (IC ₅₀ for HuCCA cells = 8.7 μM, QBC-939 cells = 9.3 μM, and RBE = 8.9 μM)
[31]	<i>Derris indica</i> (Lamk.) Benn. (Leguminosae)/ <u>Candidione</u>	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]	<i>Derris indica</i> (Lamk.) Benn. (Leguminosae) <u>ethylacetate extract/ a new furanoflavonoid derivative, 4'-hydroxypinnatin (1) and 5 known compounds</u>	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]	<i>Derris indica</i> (Lamk.) Benn. (Leguminosae) <u>hexane extract/isolated Derrivanone (1) and Derrischalcone + 14 known compounds</u>	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]	<i>Derris malaccensis</i> (Benth.) Prain (Leguminosae)/ <u>Pomiferin</u> (prenylated isoflavonoid)	IC ₅₀ for HuCCA-1 cells = 0.9 μg/mL

continued

► Table 1 Continued

References	Plants/Active compounds (Family)	Antiproliferative activity
[50]	<i>Derris malaccensis</i> (Benth.) Prain (Leguminosae)/ <u>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</u>	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]	<i>Dioscorea membranacea</i> 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]	<i>Garcinia hanburyi</i> 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]	<i>Garcinia hanburyi</i> Hook.f. (Clusiaceae)/4 caged xanthenes: <u>isomorellin, isomorellinol, forbesione gambogic acid</u>	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]	<i>Garcinia hanburyi</i> Hook.f. (Clusiaceae)/ <u>isomorellin</u>	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]	<i>Holothuria scabra</i> Jaeger (sea cucumber)/ <u>Scabraside D</u> (sulfated triterpene glycoside)	Significant activity against CL6 cells (IC ₅₀ = 12.8 µg/mL at 24 h)
[9]	<i>Kaempferia galanga</i> L. (Zingiberaceae) ethanol extract/ <u>Ethyl-p-methoxycinnamate (EPMC)</u>	Extract and EPMC: moderate activity against CL6 cells (IC ₅₀ = 64.2, 49.19 µg/mL; SI = 2.2, 2.09)
[10]	<i>Kaempferia galanga</i> L. (Zingiberaceae) ethanol extract/ <u>Ethyl-p-methoxycinnamate (EPMC)</u>	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]	<i>Kaempferia parviflora</i> Wall. ex Baker (Zingiberaceae) (crude ethanol extract)/ <u>5,7,4-trimethoxyflavone (KP.8.10)</u>	Flavonoid component in <i>K. parviflora</i> 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]	<i>Mylabris phalerata</i> (Pallas) or <i>Mylabris cichorii</i> (Laeus)/ <u>Cantharidin, Norcantharidin</u>	Cantharidin: most sensitive (IC ₅₀ : RBE cells = 2 µM, QBC939 cells = 3 µM, HCCC9810 cells = 3 µM)
[55]	<u>Phenformin and Quercetin and Myricetin</u> (from several plant species)	Quercetin: enhancement of activity of phenformin against KKU-256 cells (IC ₅₀ = 1363 µM)
[56]	<i>Phomopsis archeri</i> B. Sutton (<i>fungus</i>)/ <u>phomoarcherins A–C (sesquiterpenes), kampanol A, R-mevalonolactone, ergosterol, ergosterol peroxide</u>	Compounds 1–4: IC ₅₀ = 0.1–19.6 µg/mL (KKU-100, KKU-M139, KKU-M156, KKU-M213, and KKU-M214 cells)
[57]	<i>Pinellia ternata</i> (Thunb.) Makino (Araceae)/ <u>Banxia: polysaccharide (PTPA)</u>	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]	<i>Piper longum</i> L. (Piperaceae)/ <u>Piperlongumine</u>	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]	<i>Piper longum</i> L. (Piperaceae)/ <u>Piperlongumine</u>	IC ₅₀ for HuCCT-1–1 cells = 24.8 and 4.2 µM at 24 and 48 h, respectively
[7]	<i>Piper nigrum</i> L. (Piperaceae)/ <u>Piperine, Piperine-free Piper nigrum (black pepper) dichloroqmethane extract (PFPE)</u>	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]	<i>Pistacia atlantica</i> Desf. (Anacardiaceae)/ <u>Mastic gum resin</u>	Activity against KMBC cells: IC ₅₀ = 15.34 µg/mL
[32]	<i>Plumbago indica</i> L. (Plumbaginaceae)/ <u>Plumbagin</u>	IC ₅₀ for CL6 cells = 24.00 µM, SI = 2.28 (low)

continued

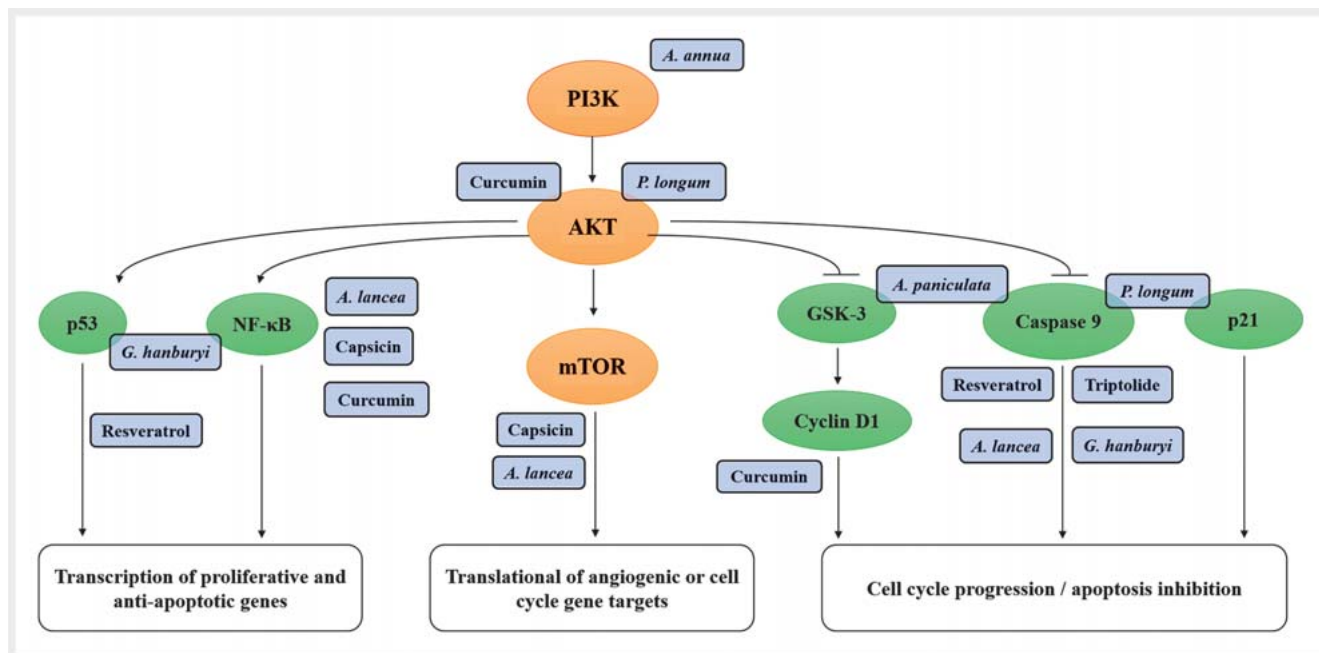
► **Table 1** *Continued*

References	Plants/Active compounds (Family)	Antiproliferative activity
[30]	<i>Reseda luteola</i> L. (Resedaceae)/ <u>Luteolin</u>	Potent activity against KKU-M156 cells (IC ₅₀ = 10.5 and 8.7 μM at 24 and 48 h, respectively)
[16]	<i>Rhinacanthus nasutus</i> (L.) Kurz (Acanthaceae)/ <u>Rhinacanthin-C</u>	Potent activity against KKU-M256 cells (IC ₅₀ = 1.50 μM)
[59]	<i>Tanacetum parthenium</i> L. (Compositae)/ <u>Parthenolide</u>	IC ₅₀ for SCK cells = 10 μM
[23]	<i>Tiliacora triandra</i> (Colebr.) Diels (Menispermaceae)/ <u>Tiliacoronine</u>	Significant activity against KKU-M055, KKU-100, KKU-M213, and KKU-M214 cells (IC ₅₀ = 4.5–7 μM)
[13]	<i>Trichosanthes cucumerina</i> L. (Cucurbitaceae)/ <u>Cucurbitacin B (CuB)</u> (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]	<i>Tripterygium wilfordii</i> Hook. f. (Celastraceae)/ <u>Triptolide</u>	Potent activity against HaLCCA-1.1, HaLcca-2, HaTCCA-1.1 cells (IC ₅₀ = 0.05 mg/mL for all cells)
[15]	<i>Tripterygium wilfordii</i> Hook. f. (Celastraceae)/ <u>Triptolide</u>	IC ₅₀ for HuCCT1, QBC939, and FRH0201 cells = 12.6, 20.5, 18.5 nM at 48 h, respectively
[11]	<u><i>Zingiber officinale</i> Roscoe (ginger) (Zingiberaceae) ethanol extract</u>	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]	<u><i>Atractylodes lancea</i> (Thunb.) DC. (Compositae) ethanol standardized extract (CMC capsule formulation)</u>	Clinical study: Phase I study, 48 healthy participants. Thais: <i>Group 1</i> : single oral dose of 1000 mg of <i>A. lancea</i> or placebo (20:4 participants). <i>Group 2</i> : daily oral doses of 1000 mg <i>A. lancea</i> 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]	<u><i>Atractylodes lancea</i> (Thunb.) DC. (Compositae) ethanol standardized extract (CMC formulation)/β-eudesmol and atractylodin</u>	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 <i>A. lancea</i> (Thunb.) DC. and compounds in complement with the direct action on apoptosis induction. Atractylodin: significant inhibition of IL6, TNF-α; <i>A. lancea</i> 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. <i>A. lancea</i> (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]	<u>PHY906 formulation</u>	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/synthetic 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 ($IC_{50} > 100 \mu\text{g/mL}$ for the herbal extract and $> 100 \mu\text{M}$ for the isolated compounds/synthetic analogs), (ii) moderate activity ($IC_{50} 10\text{--}100 \mu\text{g/mL}$ for the herbal extract and $10\text{--}100 \mu\text{M}$ for the isolated compounds/synthetic analogs), and (iii) relatively potent ($IC_{50} < 10 \mu\text{g/mL}$ for the herbal extract and $< 10 \mu\text{M}$ 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.: $IC_{50} < 1 \mu\text{M}$] [13–15], followed by rhinacanthin C [from *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae): $IC_{50} = 1.5 \mu\text{M}$] [16], compounds from *D. membranacea* Pierre ex Prain & Burkill: $IC_{50} = 1\text{--}2 \mu\text{M}$] [8], andrographolide and analogs [from *A. paniculata* (Burm.f.) Nees: $IC_{50} = 3 \mu\text{M}$] [17–19], cantharidin and norcantharidin [from *Mylabris phalerata* (Pallas): $IC_{50} = 2\text{--}3 \mu\text{M}$] [20], isolated/synthetic compounds from *Atalantia monophylla* DC. (Rutaceae): $IC_{50} = 3\text{--}5 \mu\text{M}$] [21, 22], tiliacoricinone [from *Tiliacora triandra* (Colebr.) Diels (Menispermaceae): $IC_{50} = 4\text{--}7 \mu\text{M}$] [23]. Curcumin and analogs (Zingiberaceae): $IC_{50} 3\text{--}17 \mu\text{M}$] [24–27], piperlongumine [from *P. longum* L.: $IC_{50} = 4\text{--}15 \mu\text{M}$] [28, 29], luteolin [from *Reseda luteola* L. (Resedaceae): $IC_{50} = 10 \mu\text{M}$] [30], candidione [from *Derris indica* (Lamk.) Benn.: $IC_{50} = 12\text{--}17 \mu\text{M}$] [31], and plumbagin [from *Plumbago indica* L. (Plumbaginaceae): $IC_{50} = 24 \mu\text{M}$] [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 IC₅₀ 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 G₁ 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 phar-

macokinetics 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 antiproliferative activities against human CCA cells with IC₅₀ 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 G₂/M phase [66]. The action of curcumin in CCA involves multiple molecular targets/signaling pathways, including transcription factors (NFκB, STAT3, and AP1), peroxisome proliferator-activated receptor, AKT activation pathway, B-cell lymphoma 2, B-cell lymphoma-extra large, cell survival proteins (cIAP1, cIAP2, and survivin 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 xanthenes (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 IIa trial in patients with advanced malignant tumors, i.e., lung, gastrointestinal, liver, breast, and renal adenocarcinoma [140]. Nevertheless, no clinical study was conducted in patients with advanced-stage CCA. The antiproliferative activity of both the extract (IC₅₀ = 2–3 µg/mL) and isolated compounds/synthetic analogs (IC₅₀ = 0.03–3 µM) is considered potent [6, 51]. The extract and caged xanthenes induce apoptosis via the mitochondrial pathway [51] and induction of G₀/G₁-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, gambogic 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 μ M) [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, PI3K3, 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 (NF κ B, 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 eIF2 α) [142]. *In vitro* studies showed that ginger has promising antiproliferative 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 μ M) [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_0/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_0/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 chili 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 IC₅₀ 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.

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