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•Review•

Ethnopharmacology, chemodiversity, and bioactivity of *Cephalotaxus* medicinal plants

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[ABSTRACT] *Cephalotaxus* is the only genus of Cephalotaxaceae family, and its natural resources are declining due to habitat fragmentation, excessive exploitation and destruction. In many areas of China, folk herbal doctors traditionally use *Cephalotaxus* plants to treat innominate swollen poison, many of which are cancer. Not only among Han people, but also among minority ethnic groups, *Cephalotaxus* is used to treat various diseases, e.g., cough, internal bleeding and cancer in Miao medicine, bruises, rheumatism and pain in Yao medicine, and ascariasis, hookworm disease, scrofula in She medicine, etc. Medicinal values of some *Cephalotaxus* species and compounds are acknowledged officially. However, there is a lack of comprehensive review summarizing the ethnomedicinal knowledge of *Cephalotaxus*, relevant medicinal phytometabolites and their bioactivities. The research progresses in ethnopharmacology, chemodiversity, and bioactivities of *Cephalotaxus* medicinal plants are reviewed and commented here. Knowledge gaps are pinpointed and future research directions are suggested. Classic medicinal books, folk medicine books, herbal manuals and ethnomedicinal publications were reviewed for the genus *Cephalotaxus* (Sanjianshan in Chinese). The relevant data about ethnobotany, phytochemistry, and pharmacology were collected as comprehensively as possible from online databases including Scopus, NCBI PubMed, Bing Scholar, and China National Knowledge Infrastructure (CNKI). "*Cephalotaxus*", and the respective species name were used as keywords in database search. The obtained articles of the past six decades were collated and analyzed. Four *Cephalotaxus* species are listed in the official medicinal book in China. They are used as ethnomedicines by many ethnic groups such as Miao, Yao, Dong, She and Han. Inspirations are obtained from traditional applications, and *Cephalotaxus* phytometabolites are developed into anticancer reagents. Cephalotaxine-type alkaloids, homoerythrina-type alkaloids and homoharringtonine (HHT) are abundant in *Cephalotaxus*, e.g., *C. lanceolata*, *C. fortunei var. alpina*, *C. griffithii*, and *C. hainanensis*, etc. New methods of alkaloid analysis and purification are continuously developed and applied. Diterpenoids, sesquiterpenoids, flavonoids, lignans, phenolics, and other components are also identified and isolated in various *Cephalotaxus* species. Alkaloids such as HHT, terpenoids and other compounds have anticancer activities against multiple types of human cancer. *Cephalotaxus* extracts and compounds showed anti-inflammatory and antioxidant activities, immunomodulatory activity, antimicrobial activity and nematotoxicity, antihyperglycemic effect, and bone effect, etc. Drug metabolism and pharmacokinetic studies of *Cephalotaxus* are increasing. We should continue to collect and sort out folk medicinal knowledge of *Cephalotaxus* and associated organisms, so as to obtain new enlightenment to translate traditional tips into great therapeutic drugs. Transcriptomics, genomics, metabolomics and proteomics studies can contribute massive information for bioactivity and phytochemistry of *Cephalotaxus* medicinal plants. We should continue to strengthen the application of state-of-the-art technologies in more *Cephalotaxus* species and for more useful compounds and pharmacological activities.

[KEY WORDS] *Cephalotaxus*; Ethnomedicine; Chemodiversity; Alkaloids; Bioactivity; Pharmacology
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Introduction

The genus *Cephalotaxus*, the sole member of Cephalotaxaceae family [1], has at least nine species worldwide. *Cephalotaxus* plants are distributed in the south of East Asia and Indochina Peninsula; there are at least eight species in China, five of which are endemic. They are scattered in the provinces to the east of Hengduan Mountains, from Qinling to Dabie Mountains and south of Jiangsu Province, as well as

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Taiwan. They are born in the broad-leaved forest in the low mountains to the middle mountains with an altitude of 200−1900 m from the subtropical to the northern tropics. Only *C. fortunei var. alpina* H. L. Li [2, 3] is born in the subalpine with an altitude of 2300−3700 m. The most widely distributed and abundant resources are *C. fortunei* Hook. (Fig. 1) [4] and *C. sinensis* (Rehder & E. H. Wilson) H. L. Li (Fig. 2) [5], while those with narrow distribution and few plants are endangered *C.lanceolata*K.M.FengexC.Y.ChengW.C.Cheng&L.K.&L.K. Fu (Fig. 3) [3] in Gongshan, Yunnan Province, and *C. hainanensis* H. L. Li [6, 7]. The *Cephalotaxus* plants are extensively studied for the anticancer *Cephalotaxus* alkaloids and diterpenoids [8]. There are also many other potentially useful phytometabolites in different species [9, 10], which are found to possess diverse bioactivities and pharmacological potencies [11, 12].

The family Taxaceae consists of five genera (not including *Cephalotaxus*) and at least 35 species [1], and the most closely related family is Cephalotaxaceae. Unlike medicinally important *Taxus* [13, 14], there is a lack of comprehensive review about the latter, and how the *Cephalotaxus* drug development benefit from ethnomedicine is seldom known. In 1950s, an American scholar found that *Cephalotaxus wilsoniana* Hayata, native to Taiwan, could have anticancer activity, but the research did not go well. Later Doctor Mingji Pan of Fujian Province met a young man of Jianyang Pharmaceutical Factory, who recommended *Cephalotaxus* to Dr. Pan for its anticancer potency. In the course of clinical practice, Dr. Pan also saw the root of *Cephalotaxus* purchased by the patient from the herbalist. After the patient took *Cephalotaxus* root, a series of reactions appeared, which attracted Pan's attention. According to the instructions of the herbal peasant, the cancer patient picked up two *C. fortunei* roots with 3 cm diameter and 10 cm length, which were sliced and decocted with lean pork for 1 hour, and two bowls of thick herbal juice were taken twice. However, severe gastrointestinal discomfort, nausea, vomiting and diarrhea followed. Dr. PAN saw from the adverse reactions of patients that *Cephalotaxus* has cytotoxic effect, which is the common reaction after western style chemotherapy. Then Pan cooperated with Jianyang Pharmaceutical Factory to purify *C. fortunei* and make it into an injection. After many experiments on tumor bearing mice, he found a safe and effective dose, enabling the mouse tumor significantly reduced. Later, *C. fortunei* preparation was developed by Fuzhou Pharmaceutical Factory of Fujian Province, and Pan was still responsible for clinical trials. He found that *C. fortunei* had certain effects on malignant lymphoma, leukemia, lung cancer, pancreatic cancer, liver cancer, and gastric cancer, etc. These studies attracted the attention of the Ministry of Health. At that time, during the Cultural Revolution (1966−1976), a "National research cooperation group of *Cephalotaxus*" was established with Fujian as the team leader. Dr. Pan continued to preside over the research work of anticancer *C. fortunei*. After years of efforts, it was determined that the most prominent clinical effect is from extracted monomers, i.e., homoharringtonine (HHT) and harringtonine (HT). HHT becomes one of the best medicines for the treatment of non-lymphocytic leukemia, especially acute monocytic leukemia.

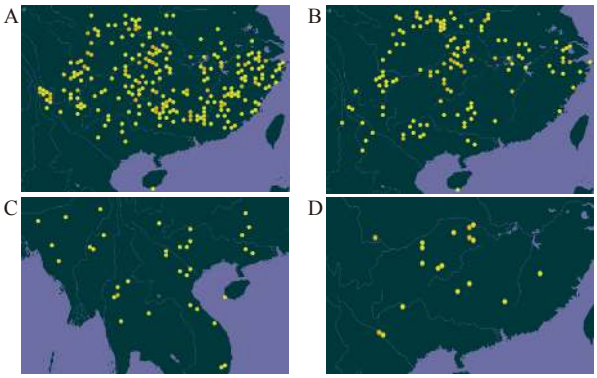


Fig. 1 Geographic distribution of A, *Cephalotaxus fortunei*; B, *C. sinensis*; C, *C. mannii*; D, *C. oliveri*

Fig. 2 *Cephalotaxus sinensis*, taken in South China Botanical Garden, Guangzhou, China

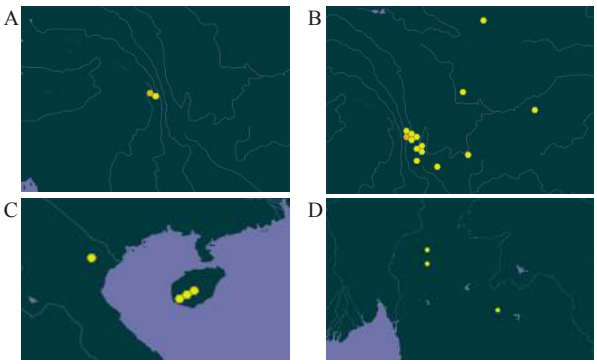


Fig. 3 Geographic distribution of A, *Cephalotaxus lanceolata*; B, *C. fortunei var. alpina*; C, *C. hainanensis*; D, *C. griffithii*

This review briefly summarizes the ethnomedicinal uses of various *Cephalotaxus* species and recent progresses in chemodiversity and phytochemistry, as well as bioactivities and pharmacology of *Cephalotaxus* medicinal plants. Traditional knowledge about various uses of *Cephalotaxus* should be further mined and collated. More chemical and pharmacological studies should be conducted to validate/rationalize traditional uses and expand medicinal values. In order to improve the production of secondary metabolites in *Cephalotaxus*, the following aspects, e.g., molecular biology, genetics

and evolution of this genus, biosynthesis and metabolism of useful phytometabolites, as well as the use of various omics platforms, should also be strengthened.

Ethnobotany and Ethnopharmacology

In many areas of China, folk herbal doctors traditionally use *Cephalotaxus* plants to treat innominate swollen poison (many of which are cancer). Not only among Han people, but also among minority ethnic groups, *Cephalotaxus* is used to treat various diseases. In Miao medicine *Cephalotaxus* is called as Feibajiu or Xuebamu literal meaning: blood Baba wood), etc. Its twigs or whole plant is used in treating internal bleeding and cancer (Yunnan Medicine Record and Ethnomedicine II, http://www.zhouzhiyuan.com/archives/2266). Miao nationality in western Hunan uses the stem bark and twigs to treat malignant tumor and cough. In Yao medicine of Guangxi Zhuang Autonomous Region, *Cephalotaxus* is called as Ke'E. Its roots, bark and leaves are used to treat bruises, rheumatism and pain. In Dong medicine of central-south and southwest China, *Cephalotaxus* is called as Tongbiansui, and its roots, bark and leaves are used to treat trauma, injuries and rheumatism. In She medicine of southeast China, *Cephalotaxus* is called as Gouweisong (dog tail pine), Taosong (peach pine), Jiansong (sharp pine), Yefeizi (wild *Torreya grandis*), Hushanshu (tiger fir), or Yanshan (rock fir), etc. Its roots, stems, seeds and leaves are used for ascariasis, hookworm disease, scrofula (lymphotuberculosis, lymphoma, etc.), and cancer. In folk medicine, people also use the method of stewing lean pork with *Cephalotaxus*.

The National Herbal Medicine Collection [15] was born in the 20th century when the Chinese herbal medicine mass movement was launched. It is the first large-scale Chinese herbal medicine reference book and one of the representative works of modern materia medica since the founding of People's Republic of China. Four *Cephalotaxus* species, i.e., *C. fortunei*, *C. hainanensis*, *C. oliveri* Mast. and *C. sinensis* are listed in this official book. Seeds, branches and leaves of *C. fortunei* are traditionally used as medicine. Seeds can be picked in autumn, while branches and leaves can be picked in four seasons. As for the drug nature (Yao Xing) and flavor, the seed is sweet, astringent and plain; branches and leaves are bitter, astringent and cold. The seed is used as parasite repellent and for eliminating the accumulation of food; branches and leaves are used in anticancer. The total alkaloid of *C. fortunei* has a good effect on lymphosarcoma and lung cancer (detailed below). The usage and dosage of seeds are as follows: 5−6 Qian are decocted in water, which are orally taken once before breakfast and supper respectively; or they are stir-fried before serving. As for alkaloid of branches and leaves, 2 ± 0.5 mg/kg body weight per day are administered in adults, and the intramuscular injection is conducted twice a day. *C. hainanensis*, *C. oliveri* and *C. sinensis* are also used to combat against cancer in various regions, e.g., *C. hainanensis* is used as a kind of Li medicine in Hainan Province of south China. As early as the 1970s, researchers studied the anticancer active ingredients of eight *Cephalotaxus* species in China. *C. hainanensis* had the highest content of ester alkaloids, up to 11 of which were identified.

The similar ethnomedicinal use of *C. fortunei* is also recorded in Great Dictionary of Chinese Medicine [16]. In Chinese Materia Medica [17], the potential toxicity of branches/leaves of *C. fortunei* is highlighted. It is recorded that in Hunan Province, the postpartum abdominal distention can be treated with a herbal formula, which consists of 9 g of branches/leaves of *C. fortunei*, 9 g of Si Mian Feng (tetrahedral wind), 9 g of Yan Fu Zi (rock aconite), 4.5 g of areca, 9 g of hawthorn, 6 g of Dang Xu, 6 g of *Akebia* stem (Mu Tong), and 6 g of Xue Pao Mu (blood soaked wood).

The plant morphology and fruit of *C. sinensis* (Cu Fei, coarse *Torreya*) are similar to *Torreya grandis* Fortune ex Lindl. (Taxaceae), but the wood texture is coarser than the latter. Its seeds are also used as substitutes for *T. grandis*, which is called indigenous Chinese torreya. The anticancer use of *C. sinensis* recorded in Chinese Materia Medica is similar to that of *C. fortunei*.

Chemodiversity

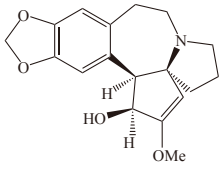
Alkaloids

Cephalotaxine (CET)-type alkaloids (Fig. 4), homoerythrina-type alkaloids (Fig. 5), other alkaloids (Fig. 5) and HHT are abundant in *Cephalotaxus*. CET-type alkaloids include oxygenated alkaloids, aromatic ester-type alkaloids, cephalezomines and related alkaloids, dimeric alkaloids, CET N-oxides and isocephalotaxine, cephastigiamide, and cephalocyclidin, etc. [18]. *Cephalotaxus* alkaloids have been known as a family of plant secondary metabolites for more than 60 years. Extracts of *Cephalotaxus* showed significant activity against leukemia in mice, leading to the upsurge of chemical research. CET (Fig. 1), the representative alkaloid of this series, was isolated from *C. drupacea* Siebold & Zucc. in 1963 [19]. The subsequent revelation of promising anticancer activity among new *Cephalotaxus* derivatives triggered extensive structure elucidation and biological studies in this family. The main structural feature of this cephalotaxane family is the tetracyclic alkaloid backbone; it consists of an azaspiranic 1-azaspiro[4.4]nonane unit (rings C and D) and a benzazepine ring system (rings A and B), which is linked by its C3 alcohol function to a chiral oxygenated side chain by a carboxylic function alpha to a tetrasubstituted carbon center. HHT, also known as omacetaxine, is a natural alkaloid in *Cephalotaxus* and a promising drug used for the treatment of chronic or accelerated phase chronic myeloid leukemia (CML) [20]. These alkaloids are solely found in the *Cephalotaxus* genus. Unlike taxanes, cephalotaxane has not been detected in other plant genera or microbial endophytes.

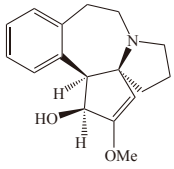
Interestingly, after a latency period of two decades, the *Cephalotaxus* alkaloids reemerge as a prolific source of new medicinal compounds. To date, more than 100 alkaloids have been identified and characterized, which are mainly classi-

fied into two structural types, i.e., homoerythrina and CET type, and the latter demonstrated remarkable antitumor activities [2]. The side chains play an important role in the anticancer activity of compounds possessing H-3 α-configuration (e.g., HHT). As an exception, cephalezomine G (Fig. 4) has the H-3 β-configuration. Homoerythrina and CET have the same biosynthetic origin. Most homoerythrina type alkaloids have the H-3 α-configuration, suggesting that there are more CET type alkaloids with this configuration. Synthetic studies also made progresses in the past 20 years [19], and various methods/schemes have been developed to obtain the first semisynthetic HHT of high purity suitable for leukemia therapy, followed by enantiomerically pure CET, HHT, and related alkaloids.

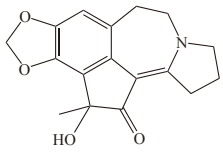
I. CET related alkaloids



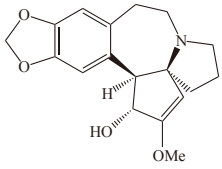
(**1**) Cephalotaxine (3*S*, 4*S*, 5*R*)



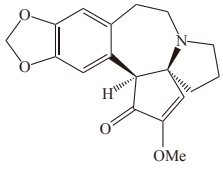
(**2**) Cephalotaxane



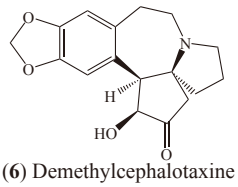
(**3**) Hainanensine



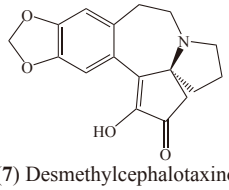
(**4**) Epicephalotaxine



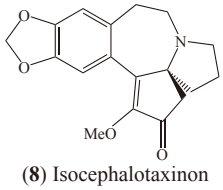
(**5**) Cephalotaxinone



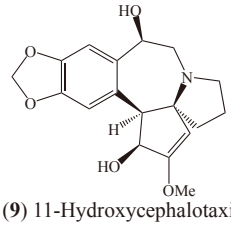
(**6**) Demethylcephalotaxine



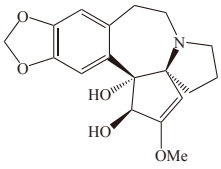
(**7**) Desmethylcephalotaxinone



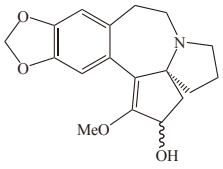
(**8**) Isocephalotaxinon



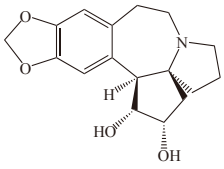
(**9**) 11-Hydroxycephalotaxine



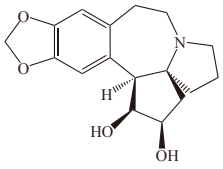
(**10**) 4-Hydroxycephalotaxine



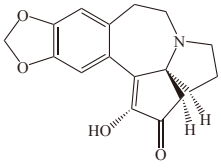
(**11**) Isocephalotaxine



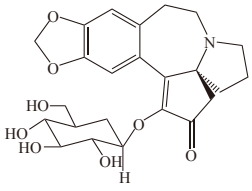
(**12**) (2*S*,3*R*)-Cephalezomine G



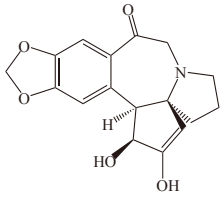
(**13**) Cephalezomine H



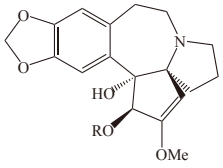
(**14**) Desmethylcephalotaxinone (stereoisomer of 12)



(**15**) Cephalezomine J

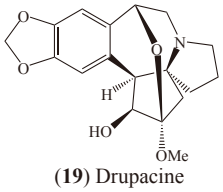


(**16**) 11-Oxodemethylcephalotaxine

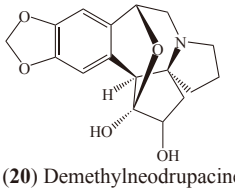


(**17**) Cephalotine C: R = O
(**18**) Cephalotine D: R = Ac

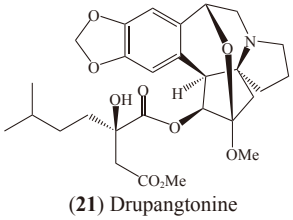
II. Oxygenated CET alkaloids



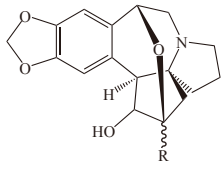
(**19**) Drupacine



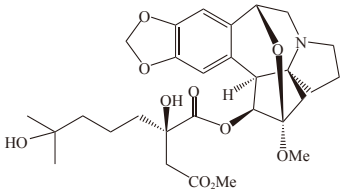
(**20**) Demethylneodrupacine



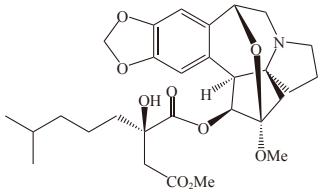
(**21**) Drupangtonine



(**22**) Cephalotine A 2α-OH
(**23**) Cephalotine B 2β-OH

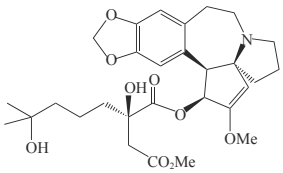


(**24**) Cephalezomine A

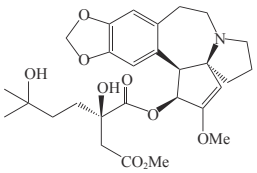


(**25**) Cephalezomine B

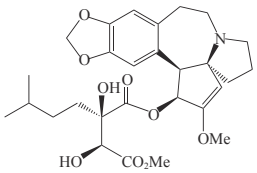
III. Ester-type CET alkaloids



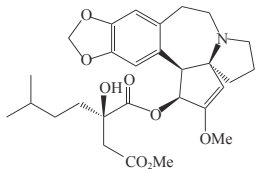
(**26**) Homoharringtonine
(2'*R*, 3*S*, 4*S*, 5*R*)



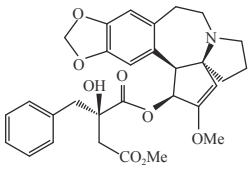
(**27**) Harringtonine
(2'*R*, 3*S*, 4*S*, 5*R*)



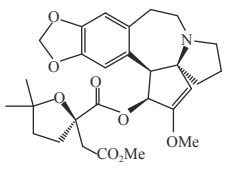
(**28**) Isoharringtonine
(2'*R*, 3*S*, 4*S*, 5*R*)



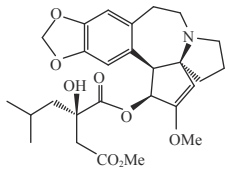
(**29**) Deoxyharringtonine
(2'*R*, 3*S*, 4*S*, 5*R*)



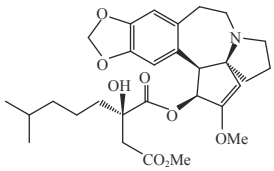
(**30**) Neoharringtonine



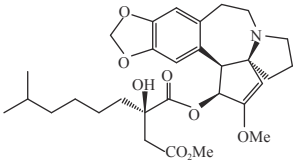
(**31**) Anhydroharringtonine



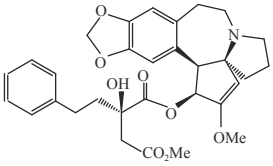
(**32**) Nordeoxyharringtonine



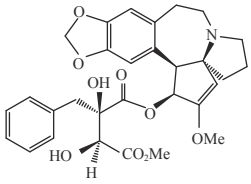
(**33**) Homodeoxyharringtonine



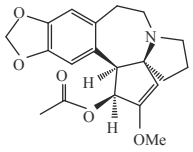
(**34**) Bishomodeoxyharringtonine



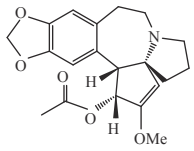
(**35**) Homoneoharringtonine



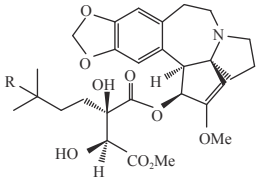
(**36**) (2'*R*,3'*S*)-Hydroxyneoharringtonine



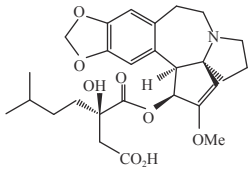
(**37**) (−)-Acetylcephalotaxine



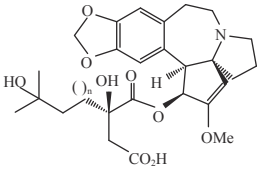
(**38**) (+)-Acetylcephalotaxine



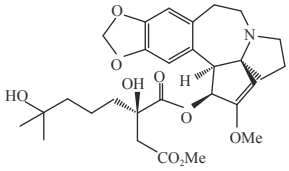
(**39**) Isoharringtonic acid R = H
(**40**) 3'*S*-Hydroxy-5'-des-*O*-methylharringtonine: R = OH



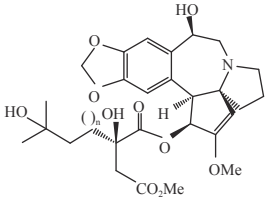
(**41**) Deoxyharringtonic acid



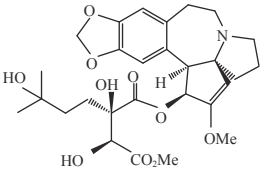
(**42**) Harringtonic acid: n = 1
(**43**) Homoharringtonic acid: n = 2



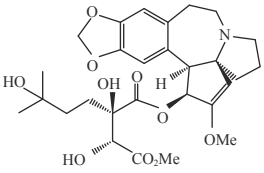
(**44**) 11α-Hydroxyhomodeoxyharringtonine



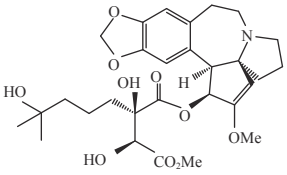
(**45**) 11β-Hydroxyhomodeoxyharringtonine: n = 2
(**46**) 11β-Hydroxydeoxyharringtonine: n = 1



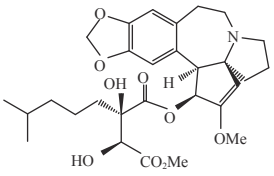
(**47**) Cephalezomine C



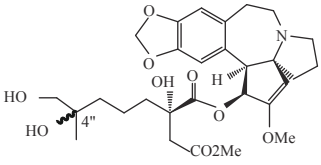
(**48**) Cephalezomine D



(**49**) Cephalezomine E

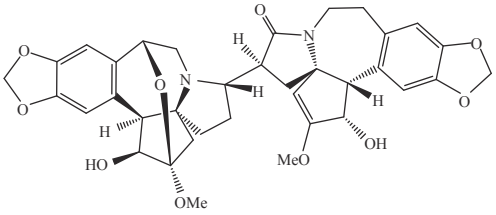


(**50**) Cephalezomine F

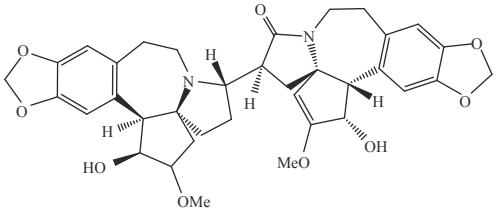


(**51**) Cephalezomine K: (epimers at C-4")
(**52**) Cephalezomine L

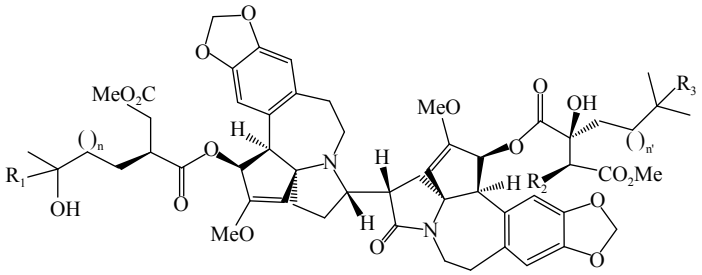
IV. Dimeric CET alkaloids



(**53**) Cephalancetine D

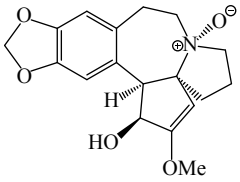


(**54**) Cephalancetine C

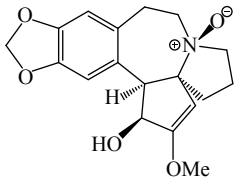


(**55**) Cephalotaxidine: $n = n' = 2$, $R_1 = R_3 = OH$, $R_2 = H$
(**56**) Bis-cephalezomine A: $n = 2$, $n' = 1$, $R_1 = R_3 = OH$, $R_2 = H$
(**57**) Bis-cephalezomine B: $n = 1$, $n' = 2$, $R_1 = R_3 = OH$, $R_2 = H$
(**58**) Bis-cephalezomine C: $n = n' = 1$, $R_1 = R_3 = OH$, $R_2 = H$
(**59**) Bis-cephalezomine D: $n = 2$, $n' = 1$, $R_1 = R_2 = OH$, $R_3 = H$
(**60**) Bis-cephalezomine E: $n = 1$, $n' = 2$, $R_1 = R_2 = R_3 = H$

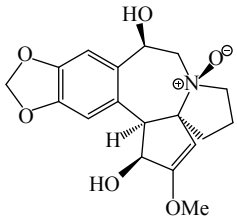
V. *N*-oxide type CET alkaloids



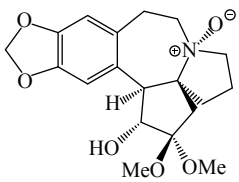
(**61**) Cephalotaxine *α N*-oxide



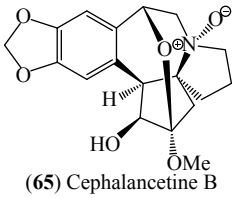
(**62**) Cephalotaxine *β N*-oxide



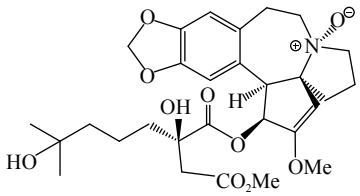
(**63**) 11-Hydroxycephalotaxine *β N*-oxide



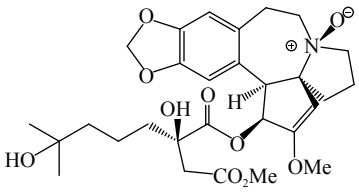
(**64**) Cephalancetine A



(**65**) Cephalancetine B

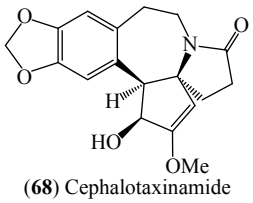


(**66**) Homoharringtonine *α N*-oxide

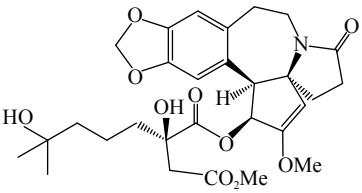


(**67**) Homoharringtonine *β N*-oxide

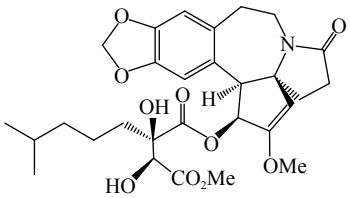
VI. Amide-type CET alkaloids



(**68**) Cephalotaxinamide



(**69**) Homoharringtonamide



(**70**) Cephastigiamide A

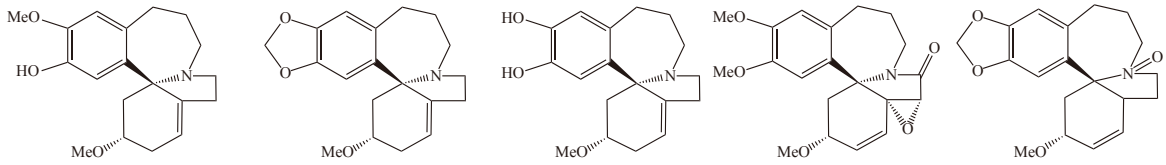
Fig. 4 Examples of cephalotaxine (CET) alkaloids from *Cephalotaxus* genus (compounds No. 1−70)

C. lanceolata and C. fortunei var. alpina

C. lanceolata (Yunnan plum yew) is an understorey shrub in woodlands, and is scattered in broad-leaved forests of northwest Yunnan (Fig. 3), China at elevations around 1900 m. *C. fortunei var. alpina* is a poorly known variety from the mountains in southwest China. Deforestation is likely to have had an impact within its restricted range and it has been assessed as Near Threatened. Five new alkaloids (Fig. 4), together with 24 known ones were isolated from leaves and twigs of *C. lanceolata* and *C. fortunei var. alpina* [2]. Cephalotine A and B are oxygenated CET alkaloids, cephalotine C and D are ordinary CET alkaloids, and cephalotine E (Fig. 5) is homoerythrina alkaloid. The known alkaloids were drupacine, cephalotaxinone, acetycephalotaxine, cephalezomine J, desmethylcephalotaxine, isocephalotaxinone, 1l-hydroxycephalotaxine, CET, lucidinine, comosidine, schelhammeridine, 3-epischelhammeridine, comosine, 3-epicomosine, 3-epischelhammericine, fortunine, taxodine, *O*-methylschlammericine, cephalezomine M, homoisoharringtonine, HHT, isoharringtonine, epidesoxyharringtonine, and desoxyharringtonine.

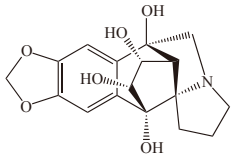
Three new alkaloids, cephalancetines A, B, and D, together with 10 known alkaloids, were isolated from branches and leaves of *C. lanceolata* [21]. Cephalancetine A and B are *N*-oxide type CET alkaloids (Fig. 4), while cephalancetine D and the known cephalancetine C are dimeric CET alkaloids. The structures of alkaloids were elucidated based on the spectroscopic analyses, including 1D- and 2D-NMR, HR-ESI-

Homoerythrina alkaloids



(**71**) Epischelhammericine B (**72**) 3-Epischelhammericine (**73**) Cephalezomine M (**74**) 3-Epi-wilsonione (**75**) Cephalotine E

Other alkaloids



(**76**) (−)-Cephalocyclidine A

Fig. 5 Examples of homoerythrina and other alkaloids of *Cephalotaxus* (compounds No. 71−76)

MS, and single-crystal X-ray diffraction.

C. griffithii, C. hainanensis and C. mannii

C. griffithii Hook. f. is a shrub or small tree and found up to an altitude of 2000 m. It is distributed in northeast India, western Sichuan of China, and Myanmar (Fig. 3). The concentration of HT and HHT was 122.14 and 16.79 $mg \cdot g^{-1}$ CGAF (*C. griffithii* needles alkaloid fraction), respectively [22]. Treatment of K562 cells with CGAF, HT and HHT dose- and time-dependently decreased the viable cells. Interestingly, the maximum cell death was found in CGAF, with IC_{50} value 3- to 4.6-fold lower than those of HT and HHT. Alkaloids other than HT and HHT in CGAF might be predominantly responsible for K562 cell death.

C. hainanensis is endemic to China's Hainan Island (Fig. 3). It has anti-leukemia properties and is widely used in China. Exploitation of the bark and leaves as well as logging are a potential threat to this species. The column chromatography Sephadex LH-20 and ODS were used to isolate and purify alkaloids part of *C. hainanensis* [23]. The chemical structures were identified on the basis of physicochemical properties and spectral data. Thirteen compounds were isolated and identified as CET, isocephalotaxinone, cephalotaxinone, cephalezomine G, (2*R*, 3*S*, 4*S*, 5*S*)-2, 3-dihydroxycephalotaxane, acetylcephalotaxine, deoxyharringtonine, (*R*)-fortunine, (*S*)-fortunine, isomer of 3-*epi*-schellhammericine, 1,4 (PrINH)2-anthraquinone, 2-ethyl-n-hexyl benzoate, and *β*-sitosterol.

Thirteen alkaloids, i.e., cephamanin A−D, cephastigiamide A, deoxyharringtonine, drupacine, CET, epiephalotaxine, cephalancetine A, cephalotaxine *α*-*N*-oxide, cephalotaxine *β*-*N*-oxide, and 3-epischellhammericine, were identified from *C. mannii* Hook. f. [24].

Analysis of alkaloids

HT, a naturally occurring alkaloid isolated from *Cephalotaxus*, has anti-leukemia activity and is utilized for the clinical treatment of acute leukemia and lymphoma. Sodium periodate ($NaIO_4$) reacted with HT to generate five HT derivatives including four novel compounds [25]. They showed the antiproliferative activity against HL-60 acute promyelocytic leukemia cells, and the presence of C-5′ methyl group enhances the antiproliferative activity, as IC_{50} values of HT derivatives, including HT1 (5′-de-*O*-methylharringtonine), were at least 2000 times higher (> 100 $\mu mol \cdot L^{-1}$) than that of HT (about 47 $nmol \cdot L^{-1}$). In an indirect competitive enzyme-linked immunosorbent assay (icELISA) using a monoclonal antibody against HT (MAb 1D2), it was found that the antiproliferative activities were related to their cellular uptake. The antiproliferative activity of HT may be enhanced by the esterification of HT1 at the C-4′ carboxylic acid group. MAb 1D2 is reliable, accurate, and sensitive in detecting small amounts of HT in plant samples [26].

Due to the meager production of HT in *Cephalotaxus* species, plant tissue culture is developed to increase HT yield. Qualitative/quantitative methods of HT detection are required to screen high yield cell lines. In a one-step indirect competitive immunochromatographic assay (ICA), colloidal gold nanoparticles

conjugated with MAb 1D2 displayed their advantage in simple, rapid, and sensitive detection of HT in plant samples [27]. It takes only 15 min after dipping the strip into analytes, with detection limit of about 313 $ng \cdot mL^{-1}$. The conventional fiber pad was not used to quickly prepare chromatographic strip, and a single test only consumed 20 μL of analytes. ICA could be applied in the field study for finding new natural resources containing HT.

HPLC-DAD-ESI-MS/MS is powerful in characterizing fingerprints of *C. sinensis* (Figs. 4 and 5) extract [28], and among 18 common peaks 10 alkaloids including two new ones were identified by comparing the retention time, UV and ESI-MS/MS spectrum of each standard with those of each peak. The effects of collecting time/site, storage duration, drying approaches, and medicinal parts on plant chromatographic profiles were investigated by similarity analysis and principal component analysis (PCA). Using the reference fingerprint and markers, the optimal cultivation location, harvesting time, and medicinal part were determined. The raw

plant of *C. sinensis* from different sources can be efficiently identified by this approach, facilitating the study of alkaloids and quality control.

The solid phase extraction (SPE)-HPLC can be used to determine HT and HHT in *C. fortunei* [29]. The linear relationship of HT and HHT holds within 2.48−49.6 and 2.51−50.2 $\mu g \cdot mL^{-1}$ respectively. The average recovery (n = 5) was 95.4% and 95.1%, and RSD was 1.83% and 1.91%, respectively. The content of two alkaloids was the highest in the stem and the lowest in the leaf.

A modified chromotropic acid (1, 8-dihydroxynaphthylene-3, 6-disulfonic acid) spectrophotometry was used to determine total alkaloids in *C. hainanensis* [30]. A GC-MS method using HP-5 capillary column was developed to analyze alkaloids of *C. hainanensis* [6]. The temperature of sample inlet, ion source and quadrupole was 280, 230, and 150 °C respectively; the electron energy was 70 eV; the scanning range of ion detection mode was 50−550 *m/z*. The contents of three alkaloids in *C. hainanensis* can be determined simultaneously, and the results of methodological study conform to the relevant regulations. In the qualitative and quantitative analysis of alkaloids in various *Cephalotaxus* species, the ultra high pressure liquid chromatography (UPLC) has advantage in the simultaneous determination of HT, CET and HHT [7]. The distribution of three alkaloids in different tissues of *C. hainanensis* was analyzed. The content of CET and HHT was higher in blade than in twig, petiole and naturally exfoliated bark, while HT was the highest in twig, followed by petiole, blade and bark.

Purification of alkaloids

CET type alkaloids are the anti-cancer components in twigs, leaves, roots and seeds of *C. fortunei* (Fig. 4). The efficient purification technology of alkaloids is vital to make full use of limited botanical resources. Firstly raw materials are dried and crushed, and extracted with methanol at room temperature, followed by combination and concentration; the extract is then detected by thin layer chromatography (TLC), extracted with chloroform, ethyl acetate and n-butanol, and separated/purified by normal column chromatography, reverse column chromatography, Sephadex LH-20 column chromatography and HPLC, etc. The step-pH-gradient high-speed counter-current chromatography (HSCCC) was performed on a HSCCC instrument equipped with a 400-mL column [31], using the upper phase of ethyl acetate−*n*-hexane−water, with added 0.01% trifluoroacetic acid (TFA) as stationary phase, and the lower phase of ethyl acetate-n-hexane-water, with added 2% NH_4OH, 0.2% NH_4OH and 0.05% TFA as mobile phase. In each separation 9.3 mg drupacine, 12.8 mg fortunine, 15.9 mg wilsonine, 35.6 mg acetylcephalotaxine, 64.8 mg *epi*-wilsonine, and 130.4 mg CET were obtained from 800 mg extract, and purities were between 81.2% and 97.5%, with > 90% recovery of each alkaloid. The structures of six alkaloids were identified by ESI mass spectrum and ^1H and ^{13}C NMR. In another study, the original purity of HT and paclitaxel were 33.1% and 36.5%, respectively [32]. In hexane− ethyl acetate−methanol−ethanol−water (5 : 7 : 5 : 1 : 6.5, *V*/*V*) system, the purity of HT and paclitaxel distillates were 88.4% and 89.7% respectively by HSCCC. After recrystallization with 50% acetonitrile, the purity of HT and paclitaxel were up to 98.7% and 97.6% respectively.

The separation technology of pH zone CCC is developed based on the common HSCCC [33]. With common HSCCC, it is difficult to obtain better separation by changing the solvent proportion. With ethyl acetate petroleum ether water system and TBME water system, good separation results can be achieved in pH zone CCC. A 400 mL column was used to separate 800 mg alkaloids from the crude extract of *Cephalotaxus* alkaloids. Three alkaloids (C-homoerythrinan, CET and acetylcephalotaxine) with purity of > 95% were obtained. The scale-up of this method could be used in the industrial separation and preparation of alkaloid drug. Six alkaloids were isolated from the crude extract of *C. fortunei* by HSCCC with pH zone and preparative HPLC. Their structures were identified by ESI-MS and NMR. The inhibitory activities of purified alkaloids on human cancer cells were verified.

Terpenoids

Diterpenoids

Abietanes (Fig. S1) and troponoids are abundant in *Cephalotaxus*. Abietanes are tricyclic diterpenes, while tropones are norditerpenes (C_{19}) representing an important class of constituents in *Cephalotaxus* [18]. Ten new cephalotane-type diterpenoids, cephanolides A−J (Fig. S1), and two known analogs were isolated and characterized from *C. sinensis* [5]. Cephanolides A−C are the first examples of A-ring-contracted cephalotane-type dinorditerpenoids, and cephanolide D is an A-ring-contracted norditerpenoid. In the biosynthetic pathway, precursors of these four compounds could be the coexisting cephalotane-type troponoids, i.e., cephanolides E−J. Seventeen new 17-nor-cephalotane-type diterpenoids, fortalpinoids A−Q, were isolated from seeds of *C. fortunei var. alpina* [4]. Fortalpinoid L is the first 17-nor-cephalotane-type diterpenoid with an 8-oxabicyclo[3.2.1]oct-2-ene moiety. The absolute configuration of fortunolide A was determined, and the structure of cephinoid Q was revised to 14-*epi*-cephafortoid A by X-ray crystallography.

Twenty-eight naturally occurring *Cephalotaxus* tropone analogs, including 19 novel ones, were identified from *C. fortunei var. alpina* and *C. lanceolata* [3]. The presence of C_{20} cephinoids A−E (Fig. S1) suggested that these tropones are norditerpenoids and could derive from labdane-type diterpenoids. The SAR (structure-activity relationship) analysis suggested that the tropone moiety and lactone ring are crucial structures for their cytotoxicity.

A new abietane diterpenoid, 12-*O*-methyl-20-deoxocarnosol-3-one, and eight known abietane diterpenoids including hinokiol, martiusane, sugiol, taxusabietane A, 13-abietadien-12-one, 5,6-dehydrosugiol, torreyayunnin, and 3-acetoxyabieta-8,11,13-trien-12-ol were obtained from leaves

and twigs of *C. lanceolata* [34].

Three C_{20} troponoids, mannolides A−C, and two new *Cephalotaxus* troponoids, 6-en-harringtonolide and 10-hydroxyharringtonolide (Fig. S1), were isolated from *C. mannii* (Fig. 4) and structurally characterized by spectroscopy and X-ray crystallography [35]. Mannolides A−C have a intact carbon skeleton, cephalotane, providing new clue on the biosynthesis of *Cephalotaxus* troponoids, a rare class of anticancer C_{19} norditerpenoids. Five new diterpenoids including two *Cephalotaxus* troponoids (20-oxohainanolidol and 20*α*-hydroxyhainanolidol), two 17-nor-cephalotane-type diterpenoids (cephafortoid A and 14-epi-cephafortoid A), and an abietane-type diterpenoid (cephafortoid B, Fig. S1), along with eight known compounds were identified from twigs and leaves of *C. fortunei* [8]. The structure of 11-hydroxyhainanolidol was revised as 10-hydroxyhainanolidol (troponoid) by X-ray crystallography. Cephafortoid A and 14-*epi*-cephafortoid A are the first examples of 17-nor-cephalotane-type diterpenoids that could be the biosynthetic precursors of co-occurring troponoids. Five known *Cephalotaxus* troponoids are fortunolide B, hainanolidol, harringtonolide, 6-en-harringtonolide, and 10-hydroxyharringtonolide; the known cephalotane-type diterpenoid is mannolide A.

Four polycyclic norditerpenoids, cephalotanins A−D, representing three novel carbon skeletons with highly rigid ring systems (Fig. S1), were isolated from *C. sinensis* and structurally characterized by multiple methods [36]. Cephalotanins A and B are new skeletal norditerpenoid trilactones, while cephalotanins C and D are norditerpenoids with novel carbon skeletons. The coexisting cephalotane troponoids could be biosynthetic precursors of cephalotanins A−D.

Three new diterpenoids (a cephalotane, an abietane and a 9(10→20)-abeo-abietane) were isolated from twigs of *C. fortunei var. alpina* [37]. Fortalpinoid R is a cephalotane diterpenoid similar to mannolide C [35]. The differences between two compounds are that the C-17 carbonyl group in the latter is replaced by a methoxy group in the former, and an exocyclic double bond at C-12 of the former substitutes the $\Delta^{12, 13}$ double bond of the latter. 3*α*, 20-Epoxy-3*β*, 12-dihydroxy-abieta-8, 11, 13-triene, an aromatic abietane diterpenoid, is structurally similar to 3*α*, 20-epoxy-3*β*, 12-dihydroxy-13-methyl-ent-podocarpa-8, 11, 13-triene [38]. The planar structure of the former differs from the latter only by the replacement of 13-methyl with a 13-isopropyl. 10*α*, 12-Dihydroxy-9(10→20)-abeo-abieta-8,11,13-trien-3-one could be biosynthetically from (4aS, 6cS)-8-hydroxy-9-isopropyl-4, 4, 6c-trimethyl-1, 2, 3, 4, 4a, 5, 6, 6c-octahydrophenanthren-3-one through oxidative cleavage of C9-C10 and subsequent rearrangement. The known abietanes are hinokinol, 12-hydroxy-abieta-8, 11, 13-trien-7-one, 12-methoxyabieta-8, 11, 13-trien-3*β*-ol, and 6*α*-hydroxysugiyl methyl ether.

Sesquiterpenoids

Eleven sesquiterpenoids were isolated from 95% ethanol extract of branches and leaves of *C. lanceolata* [39]. The monocyclic sesquiterpenoids are corchoionol C, lanceolosides A-C, 9-hydroxy-4,7-megastigmadien-3-one, 9, 10-dihydroxy-4,7-megastigmadien-3-one, 5,12-epoxy-9- hydroxy-7-megastigmen-3-one, and 5,12-epoxy-6,9-hydroxy-7-megastigmen-3-one; bicyclic sesquiterpenoids are loliolide and (3*S*, 5*R*, 8*S*)-5,8-epoxy-6-megastigmadien-3,9-diol, and clovandiol is tricyclic.

Sesquiterpene X (Fig. S2), 5-hydroxy-7-methoxy-6-methylchromone, and 21 known compounds were isolated from twigs and leaves of *C. fortunei* [9]. The 23 structures were elucidated by spectroscopy (HR-ESI-MS, 1D/2D NMR, IR) and comparing with literature. The absolute configuration of sesquiterpene X was determined via electronic circular dichroism. A new bisabolane sesquiterpenoid and a new abietane diterpenoid were isolated from *C. sinensis* [40]. The identified known compounds include allohydroxymatairesinol, angustanoic acid E, epipalustric acid, lambertianic acid, grasshopper ketone, hydroxymatairesinol, (+)-nortrachelogenin, spiramongolin, tanegool, vomifoliol, (7*S*, 8*R*)-dihydro-3′-hydroxy-8-hydroxy-methyl-7-(4-hydroxy-3-methoxyphenyl)-1′-benzofuranpropanol, threo-guaiacylglycerol-*β*-*O*-4′-dihydroconiferyl ether, and pseudolarifuroic acid, etc.

Flavonoids, lignans and phenolics

3, 5, 7, 4-Tetrahydroxy-2-methoxy-3-acetonyl-flavanone was isolated from twigs of *C. fortunei var. alpina* [37]. It is a dihydroflavonoid similar to 5, 7, 4-trihydroxy-2-methoxy-3,4-flavandione-3-hydrate. Three new biflavonoids, oliverifavones A−C (Fig. S3), and quercetin and rutin, were isolated from the endangered *C. oliveri* [10](Fig. 4). A methanol extract of leaves of *C. harringtonia var. nana* (Nakai) Rehder and its ethyl acetate (EtOAc)-soluble fraction showed strong antitumor activity against A549 and HT-29 cell lines [41]. The EtOAc fraction was purified by column chromatography and HPLC to yield three novel acyl flavonoids, one biflavonoid, and 15 known compounds including phenolics, flavonoids, and biflavonoids. The new compounds were elucidated using HR-MS and two-dimensional NMR as (2*R*, 3*R*)-3-*O*-eicosanoyltaxifolin, (2*R*, 3*R*)-3-*O*-docosanoyltaxifolin, (2*R*, 3*R*)-3-*O*-tetracosanoyltaxifolin, and 6-methyl-4′, 7, 7″-tri-*O*-methylamentoflavone.

Two new lignans, one new trisnorneolignan and eight known compounds were isolated from leaves and twigs of *C. fortunei* [8] (Fig. S4). Seventeen lignans were isolated from ethanol extracts of *C. fortunei*, including arctigenin, cephafortin B, α-conidendrin, matairesinol, nortrachelogenin, epinortrachelogenin, (7′*S*)-hydroxymatairesinol, (7′*R*)-hydroxymatairesanol, (7′*S*)-hydroxyarctigenin, secoisolariciresinol, shonanin, 4, 4′-di-*O*-methylcephafortin A, 5-(3″, 4″-dimethoxyphenyl)-3-hydroxy-3-(4′-hydroxy-3′-methoxybenzyl)-4-hydroxymethyl-dihydrofuran-2-one, dihydrodehydrodiconiferyl alcohol, 7*R*, 8*S*-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan, 7*R*, 8*R*-4, 7, 9, 9′-tetrahydroxy-3, 3′-dimethoxy-8-*O*-4′-neolignan, and threo-1, 2-bis-(4-hydroxy-3-methoxyphenyl)-1, 3-propanediol [42].

A HPLC method was used to separate secondary metabolites and quantify honokiol in cell suspension cultures of *C.*

fortunei [43].

Other components

Essential oils in leaves of *C. fortunei*, *C. haningtonia*, *C. harringtonia var. drupacea* (Siebold & Zucc.) Koidz. and *C. sinensis* were quantified [44]. The content of oils obtained by hydro-distillation in these species was 0.01%−0.22% *V*/*W*. Forty seven components of essential oils were detected by GC-MS, α-pinene varied between 18.4% and 35.1%, and sesquiterpene *β*-caryophyllene was up to 22% in *C. fortunei*.

C. fortunei nuts contained about 65% crude oil [45], which is rich in unsaturated fatty acids, oleic acid and linoleic acid, with low alkaloids (0.24%−0.41%) and tocopherol, and the main triacylglycerols were dioleoyl-monolinoleoyl and triolein. The main fatty acids in seed oil of *C. fortunei* are oleic acid (c18: 1n-9, 48.23%) and linoleic acid (c18: 2n-6, 31.53%), followed by eicosenoic acid (c20: 1n-9, 11.34%) and palmitic acid (c16: 0, 6.89%), and the content of unsaturated fatty acid is more than 90% [46], which indicate that the seed oil of *C. fortunei* has high nutritional value and can be used as a new variety of plant oil. Twenty three components were identified in the volatile oil of *C. fortunei* seeds [47], e.g., oleic acid (34.53%), palmitic acid (12.04%), butyl linoleic acid (9.56%), ethyl oleate (9.31%), as well as aliphatic alkanes and terpenes. Their antibacterial and antitumor activities warrant further development and utilization.

Six trace elements Fe, Cu, Mg, Ca, Mn, Zn in *C. fortunei* were determined by flame atomic absorption spectrometry (FAAS) [48]. The samples were digested by concentrated nitric acid and determined by standard curve method. There are abundant essential trace elements in *Cephalotaxus*. Mesophyll cells generate abscisic acid in water-stressed leaves of four conifer species (*Saxegothaea conspicua*, *Podocarpus latifolius*, *C. harringtonii* (Knight ex J. Forbes) K. Koch, and *Amentotaxus formosana* H. L. Li) [49].

Bioactivities and pharmacological utilities

These activities are briefly summarized in Table S1.

Anticancer activity and cytotoxicity of alkaloids

HHT against leukemia

Harringtonine (HT) isolated from *C. hainanensis* induced apoptosis in promyelocytic leukemia HL-60 cells [50]. HT induced changes of nuclear morphology, mitochondrial membrane potential and intracellular calcium concentration in HL-60. Yet, the highlighted bioactivity of *Cephalotaxus* alkaloids is the anti-leukemia activity of HHT [19]; its curative effect is remarkable in myeloid leukemia. HHT was approved in 2009 by European Medicine Agency and by US Food and Drug Administration (FDA) in 2012. It is also included in Chinese Pharmacopoeia 2015 version. HHT inhibited ribosome protein synthesis and has been widely used in China to treat acute myeloid leukemia (AML) since the 1970s. Trial SCMC-AML-2009 was a randomized clinical study based on the previous findings that pediatric AML patients below two years old may benefit from HHT-containing chemotherapy [51]. Patients in arm B were treated with HHT-containing regimens. Among 59 patients below two years old with de novo AML, 42 achieved a morphologic complete remission (CR) after the first course, which is as good as control therapy with anthracycline. At the end of the follow-up period, 40 patients remained in CR and five underwent hematopoietic stem cell transplantation in CR. The 5-year event-free survival (EFS) was 88% ± 6.5% for arm B, which is much higher than that of control therapy. Patients with HHT had shorter durations of leukopenia, neutropenia, and thrombocytopenia and had a lower risk of infection during consolidation chemotherapy with high dosage Ara-C.

Some signaling pathways could be targeted by HHT. For instance, HHT targets Smad3 and TGF-*β* pathway to inhibit the proliferation of AML cells [52]. HHT deregulates MYC transcriptional expression by directly binding NF-*κ*B repressing factor [53]. Myosin-9, a member of the myosin super-family, is a direct interactor of HHT [54]. HHT time-dependently up-regulated the expression level of myosin-9 in both AML and CML cell lines. The induction of leukemia cell apoptosis by HHT began in 6 h and the apoptosis continued to increase for 24 h. The up-regulated myosin-9 expression level was positively correlated with increased percentage of apoptotic cells. The overexpression of myosin-9 could increase the sensitivity of leukaemia cells to the cytotoxicity of HHT and arrest cells in S and G2/M phases. HHT-induced apoptosis of HL-60 cells is associated with the down-regulation of telomerase [55]. HHT suppresses imatinib resistance via the Bcl-6/p53 pathway in CML cell lines [56].

MicroRNAs (miRNAs) modulate the cellular sensitivity to anticancer drugs. The expression of miR-370 and Forkhead box M1 (FoxM1) in 23 patients with chronic-phase CML (CML-CP) and 10 patients with blast-crisis CML (CML-BP) was examined by qRT-PCR and western blot analysis [57]. The ectopic expression of miR-370 sensitized the CML K562 cell line to HHT by targeting FoxM1, a major regulator in cell proliferation and apoptosis. miR-370 significantly promoted HHT-mediated cell apoptosis; miR-370 and HHT synergistically down-regulated FoxM1 expression. miR-370 was moderately up-regulated after HHT treatment in K562 cells.

HHT against myeloma

Multiple myeloma (MM) is intractable in most patients. HHT is approved by FDA to treat acute and chronic myeloid lymphoma, and also inhibits the AKT pathway and induces *in vitro* and *in vivo* cytotoxicity in human MM cells [58]. HHT significantly reduced Mcl-1, a crucial protein involved in myeloma cell survival, in three myeloma cell lines [59], while BH3-only proteins, e.g., Bik, Bim, and Puma, are not altered by HHT, and their expression levels depended on the cell type. HHT also reduced the c-FLIP(L/S), activated caspase-8, and induced truncated Bid. Both intrinsic and extrinsic apoptosis pathways are involved in HHT-induced apoptosis, and the resultant imbalance between Mcl-1 and BH3-only proteins could be pivotal for the apoptosis. Moreover, HHT reduced β-catenin and XIAP proteins, which also contribute to

the disease progression and resistance to chemotherapy in MM. HHT enhanced the effects of anti-myeloma drugs melphalan, bortezomib, and ABT-737. HHT could be an option for MM therapy as it can simultaneously target multiple tumor-promoting molecules.

The high proportion polyethyleneglycol (PEG) of long-circulating HHT liposomes (LCL-HHT-H-PEG) are developed [60]. The optimal formulation of LCL-HHT-H-PEG had the higher cytotoxicity against MM RPMI8226 cells than low proportion PEG of long-circulating HHT liposomes, liposome-encapsulated-HHT, micelle-HHT, and HHT in vitro. Therapeutic studies in severe combined immunodeficient (SCID) mice implanted subcutaneously with RPMI8226 cells showed the dramatic inhibition of tumor growth in LCL-HHT-H-PEG group as compared with the HHT group and other control groups. LCL-HHT-H-PEG induced the MM apoptosis in vitro and in vivo, and may be a promising nano-device to deliver HHT into MM patients.

Combined use of HHT and other drugs

The methylation inhibitor decitabine (DAC) is used to treat myelodysplastic syndrome (MDS) and AML. DAC and HHT synergistically inhibited the viability of SKM-1 and Kg-1a AML cells [61], and enhanced the inhibition of colony formation and apoptosis induction as compared with DAC alone in SKM-1 cells. The high-dose DAC and HHT significantly up-regulated caspase-3 and caspase-9 and inhibited Bcl-XL in SKM-1 cells. The effects of DAC + HHT on apoptosis may depend on regulation of multiple apoptosis-related genes. HHT had no demethylation effects, and HHT + DAC did not enhance the hypomethylation and mRNA expression of methyltransferases DNMT1, 3A and 3B in SKM-1 cells.

HHT and aclarubicin/cytarabine synergistically induced apoptosis in t(8;21) leukemia cells and triggered caspase-3 mediated cleavage of the AML1-ETO oncoprotein [62]. PI3K/AKT inhibitor LY294002 enhanced the HHT inhibition in myeloma cells adhered to stromal cells and in SCID mouse xenograft [63]. In diffuse large B-cell lymphoma and mantle cell lymphoma cells, HHT interacts synergistically with proteasome inhibitor bortezomib through Mcl-1 and NOXA-dependent mechanisms [64].

HHT against other types of malignant tumor

HHT had anticancer effects on gefitinib-resistant non-small cell lung cancer (NSCLC) cell lines *in vitro* and *in vivo* [65]. NCI-H1975 cells with epidermal growth factor receptor (EGFR) T790M mutation are more sensitive to HHT as compared with A549 cells with wild type EGFR. HHT inhibited the cell growth, cell viability and colony formation, and induced the apoptosis through mitochondria pathway. Lung cancer patients have more interleukin-6 (IL-6); the mutant EGFR and TGFβ signal require the upregulation of IL-6 through the gp130/JAK pathway to over-activate STAT3, an oncogenic protein and a potential target for cancer therapy. HHT reversibly inhibited the IL-6 induced STAT3 tyrosine 705 phosphorylation and reduced the expression of anti-apoptotic proteins. Gefitinib-resistant NSCLC xenograft tests confirmed the anticancer effect of HHT *in vivo*.

HHT regulated the alternative splicing of Bcl-x and caspase 9 through a protein phosphatase 1 (PP1)-dependent mechanism [66]. HHT dose- and time-dependently reduced the gene expressions of anti-apoptotic Bcl-xL and caspase 9b and concomitantly increased mRNA levels of pro-apoptotic Bcl-xS and caspase 9a. Calyculin A, an inhibitor of PP1, dramatically suppressed effects of HHT on the alternative splicing of Bcl-x and caspase 9, while the specific PP2A inhibitor okadaic acid did not. Overexpression of PP1 decreased the ratio of Bcl-xL/xS and increased the ratio of caspase 9a/9b. The effects of HHT on Bcl-x and caspase 9 splicing relied on PP1 activation and were enhanced by PP1 overexpression. Triple negative breast cancer (TNBC) without BRCA1/2 gene mutation or BRCAness is intractable with poor prognosis. The effect of HHT was analyzed in four cell lines of TNBC genomic categories [67]. HHT of 20−100 ng·mL^{-1} (concentration in human plasma after subcutaneous administration) inhibited the *in vitro* growth of all cell lines for > 80% after 48−72 h exposure. HHT of 100 ng·mL^{-1} substantially reduced a major TNBC survival factor, anti-apoptotic Mcl-1, after 2-h exposure in all cell lines except MDA-MB-231. Other anti-apoptotic proteins, Bcl-2, survivin and XIAP, were also significantly down-regulated. When mice were subcutaneously and bi-daily administered with 1 mg·kg^{-1} drug for seven days, the *in vivo* growth of HHT-insensitive cell line, MDA-MB-231, was inhibited by 36.5%.

In pancreatic cancer cells, HHT induced quick protein synthesis of apoptosis inhibitory PSMD11 (26S proteasome non-ATPase regulatory subunit 11) *via* activating MEK1/ERK1/2 signaling pathway [68]. Although HHT time- and dose-dependently inhibited the proliferation and growth of PANC-1 and MiaPaCa-2 cells *in vitro*, merely part of pancreatic cancer cells were induced to die through acute apoptosis. Sorafenib inhibited MEK1/ERK1/2 pathway and improved the cytotoxity of HHT in vitro and in a genetically engineered mouse model of pancreatic cancer.

TNF-related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic ligand from the TNFα family, which can be combined with agonistic anti-TRAIL receptor antibodies in cancer therapy, as most primary human tumors are resistant to TRAIL alone. In a high-throughput screening of novel reagents, HHT effectively sensitized TRAIL-resistant colorectal cancer cells to TRAIL-induced apoptosis [69]. In TRAIL-resistant RKO or HT-29 cells, HHT and TRAIL synergistically induced the apoptosis and complete depletion of tumor cells. HHT decreased the expression of anti-apoptotic Mcl-1 and cFLIP and enhanced the TRAIL-triggered activation of JNK and p38 kinases. Except RKO/sh cFLIP cells, the decline of cFLIP or Mcl-1 significantly reduced the HHT dose in HHT + TRAIL treatment. *In vivo*, HHT + TRAIL led to the strong inhibition of HT-29 tumors implanted in immunodeficient mice.

In human bone osteosarcoma U2OS cells expressing microtubule-GFP markers, HHT significantly inhibited the cell

migration, but did not affect microtubules [70]. HHT could be an anti-migrating reagent with unknown mode of action.

Anticancer activity of HHT derivative

HHT exerts its antitumor activity via inhibiting protein synthesis and promoting apoptosis. In 1990s, HHT was used as the salvage therapy of CML patients after failure of interferon (IFN)-α therapy. After the remarkable success of imatinib mesylate in CML therapy [71], Omacetaxine mepesuccinate, a semisynthetic derivative of HHT, became more important in combating relapsed or refractory CML following TKI (tyrosine kinase inhibitor) therapy [72]. The subcutaneous omacetaxine mepesuccinate was effective in CML patients with TKI (imatinib) resistance [73].

The cytostatic bis(4-hydroxycoumarin) derivative OT-55 can be combined with omacetaxine in imatinib-resistant KBM-5 R CML cells to inhibit the expression of Mcl-1 and trigger apoptosis [74]. When fms-like tyrosine kinase (FLT) 3 inhibitor is used, omacetaxine can be an adjuvant for FLT3-ITD (internal tandem duplication) AML [75]. The most common grade 1−4 adverse reactions included anemia, diarrhea, fatigue, nausea, neutropenia, thrombocytopenia, and asthenia [76]. The drug is subcutaneously and intermittently administered (14 d on/14 d off of induction; 7 d on/21 d off in maintenance).

Anticancer activity of other alkaloids

Anhydroharringtonine, deoxyharringtonine, HHT, and homodeoxyharringtonine are among the most potent anti-leukemia alkaloids of *Cephalotaxus* [77]. They were effective in multiple cell lines. Different ester chains of these alkaloids lead to differential activity against vincristine-resistant HL-60/RV+, inspiring the molecular design based on natural alkaloids to combat multi-drug resistance. The CET type alkaloid isoharringtonine inhibited breast cancer stem-like properties and STAT3 signaling [78]. The C3 hydroxy groups of CET and drupacine were acylated by paclitaxel side chain and its isomers to give a series of derivatives [79], among which VIIIa, VIIIb, IXa and IXc significantly inhibited KB (human oral squamous cell carcinoma), HCT (huamn colon cancer) and Bel (human liver cancer) cell lines. CET time and dose-dependently inhibited HepG2 proliferation [80] and increased acid vesicle organelles. CET also increased the expression of autophagy markers LC3-II and Beclin-1. A homoerythrina alkaloid, C-3-epi-wilsonione, were cytotoxic against various human cancer cell lines [81].

Anticancer activity and cytotoxicity of terpenoids

The cephalotane-type diterpenoids fortunolides A and B of *C. sinensis* had significant cytotoxicity against tumor cell lines such as A549, HL-60, KB, and HT-29 with IC_{50} 0.464−6.093 μmol·L^{-1} [5]. Some 17-nor-cephalotane-type diterpenoids of *C. fortunei var. alpina* were significantly cytotoxic against A549 and HL-60 cells [4]. Norditerpenoids of *C. lanceolata* and *C. fortunei var. alpina* had excellent cytotoxicity against human cancer cells (IC_{50} 20−0.1 μmol·L^{-1}) *in vitro* [3]. Cephinoid H (Fig. S1) at 60 ng/mL led to 49% inhibition in zebrafish, which was better than 15 μg·mL^{-1} cisplatin (22.4%). These troponoids might affect the NF-κB signaling pathway rather than binding to microtubules. A new *Cephalotaxus* troponoid, 20-oxo-hainanolidol, was cytotoxic against A-549 and HL-60 cells with IC_{50} 1.129 ± 0.057 and 0.77 ± 0.05 μmol·L^{-1}, respectively [8]. The tropone motif is essential for the anticancer activity [35].

Anticancer activity and cytotoxicity of other compounds

Medulloblastoma (MB) is a malignant brain tumor mainly found in infants and children, around 25% of which is due to upregulation of canonical Wnt pathway with CTNNB1 mutations. Among 600 natural compounds [11], ginkgetin, a biflavone of *C. fortunei var. alpina*, was found to inhibit the Wnt pathway with an IC_{50} about 5.92 μmol·L^{-1}. In SAR analysis, the methoxy group of ginkgetin was suggested to be a functional group. Ginkgetin strongly inhibited D283 and Daoy MB cells and induced G2/M arrest in the former. Ginkgetin suppressed the expression of Wnt target genes survivin, Axin2, and cyclin D1 in MB cells. The phosphorylation of β-catenin also decreased time- and concentration-dependently. Some biflavones of *C. harringtonia var. nana* had the antitumor activity [41].

The PE (petroleum ether) extract of *C. griffithii* induced the maximal cytotoxicity as compared with other extracts, and ZR751 breast cancer cells were most sensitive [82]. PE extract induced the cell cycle arrest and apoptosis; both intrinsic and extrinsic apoptotic pathways were activated. P53 was essential for the loss of ZR751 cell viability induced by PE extract. PE extract decreased hTR, hTERT, and c-Myc expressions.

C. griffithii needle essential oil (CGNO) inhibited the proliferation and migration of human cervical cancer (HCC) cells [83]. CGNO decreased the viability of HCC (ME-180, HeLa, and SiHa) cells, and induced the apoptosis of HeLa cells through mitochondria-initiated and death receptor-mediated pathways. CGNO increased mitochondrial membrane depolarization and increased the expression of caspase-3, 8, 9, and cleaved-PARP. The activity of caspase-8 and -9 was also augmented. Wound healing and transwell migration assay showed that CGNO dramatically inhibited the migration of HeLa cells to close a scratched wound and inhibited their migration through filter towards a chemotactic stimulus.

Anti-inflammatory and antioxidant activities

Anti-inflammatory activity

The norditerpenoids of *C. lanceolata* and *C. fortunei var. alpina* had almost equal anti-inflammatory activities as compared to the positive control MG132 [3]. A polycyclic norditerpenoid, cephalotanin A, strongly inhibited NF-κB with IC_{50} 4.12 ± 0.61 μmol·L^{-1} [36]. The *in vitro* anti-inflammatory activities of *C. fortunei* compounds were assayed in RAW 264.7 cells by assessing lipopolysaccharide (LPS)-induced nitric oxide (NO) production. 5-Hydroxy-7-methoxy-6-methylchromone and sesquiterpene X (Fig. S2) showed percentage inhibitions of 24% and 35.60%, respectively [9]. Apigenin-5-*O*-β-d-glucopyranoside, taxacin and 20-hydro-

xyecdysone, which are abundant in *C. fortunei*, had significant in vitro anti-inflammatory activities.

Surgery-induced epidural fibrosis after laminectomy is often troublesome, and fibroblast proliferation is one of main causes. *In vitro*, HHT inhibited fibroblast proliferation and induced apoptosis [84]. In rats, HHT suppressed epidural fibrosis after laminectomy. The expressions of two important endoplasmic reticulum (ER) stress markers (78-kDa glucose-regulated protein and C/EBP homologous protein) were increased. HHT might induce fibroblast apoptosis via ER stress signaling pathway.

Antioxidant activity

The flavone glycoside, apigenin 5-*O*-*α*-L-rhamnopyranosyl-(1→3)-beta-D-glucopyranoside (**1**), and four known flavonol glycosides (**2**−**5**), were isolated from *C. koreana* leaf [85]. The glycoside 1 showed inhibitory activity in superoxide radical scavenging assay with IC_{50} 13 μmol·L^{-1}, and had weak activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Glycosides 2−5 had impressive activity in scavenging DPPH and superoxide radicals with IC_{50} 5.7−22.3 μmol·L^{-1}. Rutin and quercetin of *C. oliveri* had excellent antioxidant activity with IC_{50} 0.02 ± 0.1 and 0.03 ± 0.06 μmol·L^{-1}, respectively [10].

Among three extracts of *C. griffithii* stem bark, the acetone extract contained the most total phenolics and flavonoids and displayed most significant antioxidant, antibacterial, cytotoxic (IC_{50} 35.5 ± 0.6 μg·mL^{-1}), and apoptotic (46.3% ± 3.6% sub-G0/G1 population) activity [86], and PE and methanol extracts had similar effects to a lesser extent. The acetone/methanol extracts and positive control ascorbic acid had similar IC_{50} in DPPH scavenging assay, while in superoxide radical scavenging assay, the activity of acetone and methanol extracts was lower than ascorbic acid. The flavonoids and phenolics were responsible for the high antioxidant, cytotoxic, and apoptotic activity.

Immunomodulatory activity

Dysregulated activation of IFN-*β* promoter, i.e., the cyclic GMP-AMP synthase-stimulator of IFN gene (cGAS-stimulator of interferon genes (STING)) pathway, by self-DNA contributes to interferonopathy and promotes autoimmune diseases. Notably, 70% ethanol extract of *C. koreana* specifically suppressed STING-induced, but not TANK-binding kinase (TBK1)- or IRF3-induced, IFN-*β* promoter activity [87]. The responsible compounds were ester alkaloids, i.e., HHT and HT, which inhibited 2′3′-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP)-induced IFN-stimulated gene expression and interaction between STING and TBK1. CET without ester side-chain had no such effects.

Antimicrobial activity, antiparasitic activity and nematotoxicity

In assessing the antimicrobial activity, nematotoxicity, and other effects of *Cephalotaxus* compounds, it is critical to assess the quality of the concerned studies, e.g., whether necessary information on the dose range tested, the minimal active concentration, and the model used are presented, whether and what controls were used, how the reported activity is linked to traditional uses, etc.

Antiviral activity

HT and HHT have potent antiviral activity [88]. *In vitro*, 5 ng·mL^{-1} HT and 10 ng·mL^{-1} HHT, but not parental alkaloid CET (50 ng·mL^{-1}), significantly inhibited replication of recombinant varicella-zoster virus (VZV)-pOka luciferase. Cells treated with 5 μmol·L^{-1} acyclovir were the positive control. HT and HHT of 10 ng·mL^{-1}, instead of 10 ng·mL^{-1} CET, strongly inhibited VZV lytic gene expression and had strong antiviral effects against a VZV clinical isolate. They could be antiviral candidates for treating VZV associated diseases.

To assess the activity of CET and HHT against flavivirus, the bovine viral diarrhea virus (BVDV) was used as a surrogate for hepatitis C virus (HCV) [89]. The anti-HBV and anti-HCV drugs, lamivudine and ribavirin, respectively, were used as the positive control. Up to 100 μmol·L^{-1} ribavirin and lamivudine did not induce cell toxicity, but dose-dependently inhibited BVDV and HBV, respectively. In the same range, CET and HHT induced toxicity in embryonic bovine trachea (EBTr) cells and failed to inhibit BVDV. However, they inhibited the HBV production at concentrations 10- to 100-fold lower than those inducing cell toxicity, thus they could be useful in hepatitis B treatment. CET inhibited the Zika virus (ZIKV) in Vero and A549 cells [12], as it may kill virus and reduce viral replication. CET suppressed ZIKV RNA and protein expression, inhibited ZIKV replication and ZIKV mRNA/protein production. CET also inhibited Dengue virus 1-4. Further investigation and development of CET-derived drugs may lead to novel anti-flavivirus reagent.

HHT presents broad antiviral activity *in vitro* and *in vivo*. It completely inhibited infections of porcine epidemic diarrhea virus (PEDV), Newcastle disease virus (NDV), and vesicular stomatitis virus (VSV) at 500, 100, and 50 nmol·L^{-1} in cell cultures, respectively [90]. HHT of 0.2 and 0.05 mg·kg^{-1} significantly reduced viral load and alleviated severe symptoms in NDV- and PEDV-infected animals respectively. HHT moderately inhibited avian influenza virus (AIV) infection, and it has potency against various RNA viruses. HHT actively inhibited herpes simplex virus type 1 (HSV-1) replication with IC_{50} 139 nmol·L^{-1}; 1000 nmol·L^{-1} HHT led to decrease of three orders of magnitude. HHT suppressed the phosphorylation of endogenous and exogenous eukaryotic initiation factor 4E (p-eIF4E), which could regulate the selective translation of specific mRNA.

Nematotoxicity

A crude alkaloid extract of *C. fortunei* twigs and leaves contained drupacine and displayed nematotoxicity [91]. The ED_{50} of drupacine for *Bursaphelenchus xylophilus* and *Meloidogyne incognita* was 27.1 and 76.3 μg·mL^{-1} respectively. Immersion of *M. incognita* eggs in 1.0 mg·mL^{-1} crude alkaloid extract reduced hatch by 36%; immersion of second-stage juveniles (J2) led to 72%−98% immobility. Crude alkaloid extract and drupacine inhibited protease activity in extracts of the microbivorous nematode *Panagrellus redivivus*

by 50% and 80%, respectively. Application of 0.02−0.5 mg·mL^{-1} alkaloid extract to soil with *M. incognita* inoculum did not significantly reduce pepper plant shoot length or weight, as compared with nematode-inoculated and water-treated controls; 0.5 mg·mL^{-1} alkaloid extract decreased the number of eggs and J2 per root system by 69% and 73% respectively.

Antifungal activity

The norditerpene (+)-harringtonolide of *C. harringtonia var. drupacea* had cytotoxic and antifungal activities [92].

Antiparasitic activity

The parasite *Trypanosoma brucei*, causing Chagas disease, did not resume growth after HHT pretreatment [93]. The protein biosynthesis was time- and concentration-dependently inhibited. Unlike emetine, HHT is not a DNA intercalator. HHT decreased the mitochondrial membrane potential and caused cell cycle arrest, but did not affect trypanothione reductase (TryR), a crucial component of the redox system of trypanosomes. The anti-*T. cruzi* TryR potential of a skeletally diverse set of natural alkaloids was assessed computationally [94]. The preferred binding mode (low number of clusters, high cluster population) and the inferred binding interactions were used to screen the inhibitor. CET was proposed as a promising alkaloid for developing stronger and selective TryR inhibitor.

Antihyperglycemic effect

The streptozotocin (STZ)-induced diabetic rats were orally administered with aqueous extract (WtE), 80% EtOH extract (aq.EE), ethylacetate fraction (EaF) or butanol fraction (BtF) of *C. sinensis* (200 mg·kg^{-1}) for 28 d [95]. The EaF, aq.EE and BtF significantly prevented the STZ-induced elevation of serum transaminases ALT and AST, alkaline phosphatase (ALP), urea, creatinine, urine sugar and body weight, which were as potent as the standard drug tolbutamide. WtE had no significant effect. Saponins, flavonoids, terpenes, and sterols of *C. sinensis* could be responsible for the hepato-renal protection.

The antihyperglycemic effect of fractions FA, FB, FC, and FD of the 80% ethanol extract of *C. sinensis* leaves was evaluated in STZ-induced diabetic rats [96]. FC was more active than other fractions. The oral FC (0.48 g·kg^{-1}) for 10 d significantly reduced the blood glucose of diabetic rats. FC treated diabetic rats ingested significantly less food and water than 0.5% carboxymethyl cellulose-treated rats. The ethanol extract of *C. sinensis* leaves contained apigenin, apigenin-5-*O*-[*α*-L-rhamnopyranosyl-(1→4)-6-*O*-*β*-D-acetylglucopyranoside], and apigenin-5-*O*-[*α*-L-rhamnopyranosyl-(1→4)-6-*O*-*β*-D-glucopyranoside]. FC (0.1, 1, 10 mg·mL^{-1}), apigenin (0.1, 2 mg·mL^{-1}) and apigenin-5-*O*-[alpha-L-rhamnopyranosyl-(1→4)-6-*O*-beta-D-acetylglucopyranoside] (0.1, 2 mg·mL^{-1}) increased GLUT-4 transport protein in membrane preparations of mice adipocytes, which partially explain their anti-diabetes effect.

Bone effects

Bilobetin (4′-monomethylamentoflavone), sciadopitysin (amentoflavone-7,4′,4′-trimethyl ether), and 7,4′,7″,4″-*O*-methyl-amentoflavone, isolated from the EtOAc fraction of *C. koreana*, significantly increased the differentiation of mouse primary osteoblast and altered ALP activity, collagen synthesis, and mineralization [97]. SAR suggests that methoxyl groups at 4′ and 4‴ in B ring of amentoflavone-type biflavonoid could be crucial in osteoblast differentiation. Biflavonoids could be used against bone diseases such as osteoporosis.

Apigenin, isoscutellarein 5-*O*-*β*-D-glucopyranoside, kaempferol 3-*O*-*α*-L-rhamnopyranosyl(1‴→6″)-*β*-D-glucopyranoside, quercetin 3-*O*-[6″-*O*-acetyl]-*β*-D-glucopyranoside, quercetin 3-*O*-*α*-L-rhamnopyranoside, and tamarixetin 3-*O*-*α*-L-rhamnopyranosyl(1‴→6″)-*β*-D-glucopyranoside, isolated from above-ground parts of *C. koreana*, significantly inhibited osteoclast differentiation at 0.1 and 1.0 μg·mL^{-1} [98].

Other bioactivities and effects

Botanicals of agricultural utility are relatively safe, with high selectivity and easy degradation; they are promising in developing new pesticide, herbicide, and antiviral reagent. Four alkaloids of *C. sinensis* had insecticidal activity [99, 100]; they showed antifeedant and gastrotoxic effects on the 3rd instar larvae of *Plutella xylostella*, and the antifeedant and mortality rates of 1 mg·mL^{-1} CET *β*-*N*-oxide were 41% and 60% respectively after 72 h. they also showed antifeedant activity to armyworm. Eight alkaloids of *C. sinensis* inhibited tobacco mosaic virus (TMV) *in vitro*. CET and its *β*-*N*-oxide, drupacine, and 11-hydroxycephalotaxine strongly inhibited the germination of four kinds of weeds, especially *Amaranthus retroflexus*.

Patients with high-risk polycythemia vera (PV) or essential thrombocythemia (ET) who failed in or were intolerant to hydroxycarbamide or IFN-*α* therapy received HHT of 1.5 mg·m^{-2} daily by continuous infusion for seven days every month [101]. After six courses of HHT therapy, the hematological response rates were 64.7% (11/17) in PV and 72.2% (13/18) in ET. In PV, the single sign remission rates of constitutional symptoms, symptomatic splenomegaly, pruritus and bone pain were 70.0%, 77.8%, 50% and 100%, respectively. The remission rates of constitutional symptoms and symptomatic splenomegaly in ET were 66.7% and 71.4% respectively. The severe granulocytopenia and thrombocytopenia were rare, and no pancytopenia was induced. The efficacy of low-dose HHT alone on high-risk PV/ET is not lasting, so it can be used as a second-line drug for PV/ET treatment after hydroxycarbamide or IFN-*α*.

Seeds of 32 species from two major groups of gymnosperms, the ancient Cycadales and economically important Coniferales, were analyzed for inhibitors of the serine proteinases trypsin, chymotrypsin, subtilisin and elastase using isoelectric focusing (IEF) combined with gelatin replicas [102]. Several species of *Cephalotaxus*, *Pseudotsuga* and *Cycas* contained inhibitors against elastase.

Conclusion and Prospects

Through detailed database search and data collation, it turns out that four *Cephalotaxus* species are listed in the offi-

cial medicinal book in China. They are used as ethnomedicines by many ethnic groups such as Miao, Yao, Dong, She and Han, etc. Inspirations from traditional uses facilitate the R&D of anticancer (especially anti-leukemia) drug based on *Cephalotaxus* phytometabolites, including but not limited to CET-type alkaloids, homoerythrina-type alkaloids, diterpenoids, sesquiterpenoids, flavonoids, and lignans, etc [103, 104]. These promising lead compounds are abundant in *C. fortunei*, *C. sinensis*, *C. lanceolata*, *C. griffithii*, and *C. hainanensis*, etc. Newly developed methods of chemical analysis and purification are conducive to expanding the research scope of medicinal resources. More *Cephalotaxus* species and cultivars should be investigated in metabolic and pharmacological profiles. More bioactivity screening and in-depth mechanism studies should be conducted for both known compounds and newly identified components in various *Cephalotaxus* populations. Alkaloids such as HHT, terpenoids and other compounds have anticancer potency against various types of human cancer. *Cephalotaxus* extracts and compounds also display anti-inflammatory and antioxidant activities, immunomodulatory activity, antimicrobial activity and nematotoxicity, antihyperglycemic effect, and bone effect, etc. How these chemical entities are subjected to metabolic transformation and PK disposition is worth extensive explorations, as these properties are directly associated with therapeutic efficacy. Thanks to the emerging clues, we now can continue to collect and sort out folk medicinal knowledge of *Cephalotaxus* and associated organisms, so as to obtain new enlightenment to translate traditional tips into modern drugs. The combined use of different omics platforms can contribute massive information for bioactivity and phytochemistry of *Cephalotaxus* plants.

Alkaloids have been suggested for the prevention and/or management of oxidative stress and inflammation-mediated diseases [105]. Whether *Cephalotaxus* alkaloids have effects on angiogenesis is also worth studying. The angiogenesis plays a crucial role in tumor growth and invasion. Antiangiogenic compounds can be used for the control and treatment of cancers. The alkaloid-rich plants have some interesting features that effectively inhibit angiogenesis [106]. Studies about *Cephalotaxus* genetic resources, breeding, conservation, domestication, and cultivation shall be further strengthened. Most *Cephalotaxus* plants are mixed in the evergreen broad-leaved forest. The key measure to protect them is to protect the existing evergreen broad-leaved forest in different places. When utilizing the *Cephalotaxus* resources, we must pay attention to prevent overuse, and control the frequency and intensity of utilization properly to protect the ability of natural regeneration of *Cephalotaxus*. The relevant forestry and resource utilization departments should strengthen the experimental research on artificial seedling raising, introduction and cultivation [107] while collecting and comprehensively utilizing the wild resources, so as to gradually realize the sustainable utilization of artificially cultivated *Cephalotaxus*. Like *Taxus* [108, 109], *Cephalotaxus* is a species lacking in genetic research and genomic information. There are very few genetic markers that can be used effectively. The factors influencing the culture of embryo tube plantlet shall be studied, and the half sib population of *Cephalotaxus* can be obtained from the embryo and tube plantlet. Meanwhile, LC-ESI-MS technology shall be established to investigate the content differences of various alkaloids and other useful phytometabolites in the populations. The large-scale SSR marker mining of *Cephalotaxus* shall be carried out based on the public EST database, fosmid library and transcriptome data; based on EST-SSR markers, the genetic diversity analysis of sib population can be performed, and the construction of EST-SSR linkage map is based on genetic markers. The molecular breeding and selection efficiency of *Cephalotaxus* will be improved.

In the study of *Cephalotaxus* physiology, phytopathology and plant protection, *Cephalotaxus* associated microbes cannot be overlooked. Pharmacotherapy utility can be effectively mined from chemodiversity/biodiversity of these microorganisms. The identification and cloning of key enzyme genes in the medicinal compound biosynthesis pathway, determination of suitable vectors, and genetic engineering techniques to study the expression of exogenous genes will help increase the yield of useful metabolites produced by both host plants and associated microbes. Equally importantly, the *Cephalotaxus* relevant mechanization, postharvest processing, extraction, quality assurance, and analytics shall be further studied to make the technology more application-oriented.

Abbreviations

AIV: avian influenza virus; ALP: alkaline phosphatase; AML: acute myeloid leukemia; BVDV: bovine viral diarrhea virus; CET: cephalotaxine; CML-BP: blast-crisis CML; CML-CP: chronic-phase chronic myeloid leukemia; CR: complete remission; DAC: decitabine; DPPH: 1,1-diphenyl-2-picrylhydrazyl; EBTr: embryonic bovine trachea; EFS: event-free survival; EGFR: epidermal growth factor receptor; ER: endoplasmic reticulum; ESI-MS: electrospray ionization mass spectrum; ET: essential thrombocythemia; EtOAc: ethyl acetate; FLT: fms-like tyrosine kinase; FoxM1: Forkhead box M1; HCC: human cervical cancer; HHT: homoharringtonine; HSCCC: high-speed counter-current chromatography; HSV-1: herpes simplex virus type 1; HT: harringtonine; ICA: immunochromatographic assay; icELISA: indirect competitive enzyme-linked immunosorbent assay; IFN: interferon; IL: interleukin; LCL-HHT-H-PEG: high proportion PEG of long-circulating HHT liposome; MAb: monoclonal antibody; MB: medulloblastoma; MDS: myelodysplastic syndrome; miRNA: microRNA; MM: multiple myeloma; NDV: Newcastle disease virus; PCA: principal component analysis; PE: petroleum ether; PEDV: porcine epidemic diarrhea virus; PEG: polyethyleneglycol; PK: pharmacokinetic; PP1: protein phosphatase 1; PV: polycythemia vera; SAR: structure-activity relationship; SCID: severe combined immunodeficient; SPE: solid phase extraction; STZ: streptozotocin; TER: transepithelial electrical resistance; TFA: trifluoroacetic acid; TKI: tyrosine kinase inhibitor; TLC: thin layer chromato-

graphy; TNBC: triple negative breast cancer; TRA: total radioactivity; TRAIL: TNF-related apoptosis-inducing ligand; UPLC: ultra high pressure liquid chromatography; VSV: vesicular stomatitis virus; VZV: varicella-zoster virus; ZIKV: Zika virus.

Supplementary Materials

Supplementary materials are available as Supporting Information, and can be requested by sending E-mail to the corresponding author.

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