Thieme

Cnicus benedictus: Folk Medicinal Uses, Biological Activities, and *In Silico* Screening of Main Phytochemical Constituents

Authors
Katarzyna Ziętal[1], Dagmara Mirowska-Guzel[1], Alicja Nowaczyk[2], Kamilla Blecharz-Klin[1]

Affiliations
1 Department of Experimental and Clinical Pharmacology, Centre for Preclinical Research and Technology CePT, Medical University of Warsaw, Warszawa, Poland
2 Department of Organic Chemistry, Faculty of Pharmacy, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

Correspondence
Assoc. Prof. Kamilla Blecharz-Klin
Department of Experimental and Clinical Pharmacology,, Medical University of Warsaw, Centre for Preclinical Research and Technology CePT
Banacha 1B, 02-097 Warsaw, Poland
Phone: + 4 82 21 16 62 02, Fax: + 4 82 21 16 62 02
kamilla.blecharz-klin@wum.edu.pl

Keywords
Cnicus benedictus, *Centaurea benedicta*, blessed thistle, Asteraceae, cnicin, arctigenin, arctiin

received April 9, 2024
accepted after revision August 21, 2024

Bibliography
Planta Med 2024
DOI 10.1055/a-2401-6049
ISSN 0032-0943

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

ABSTRACT

Traditional medicine has long recognized the therapeutic potential of *Cnicus benedictus*, and recent scientific research has shed light on the pharmacological properties of this plant. The bioactive compounds that can be extracted from it, such as the sesquiterpene lactones arctigenin, arctiin, and cnicin, are very interesting to researchers.

In this article, based on available data from pre-clinical *in vitro* and *in vivo* studies, we delve into the pharmacology of the active constituents of this plant to explore its potential therapeutic applications and underlying mechanisms of action. In addition, we present a computer analysis designed to reveal the pharmacokinetic and toxicological properties of the main phytochemicals that are active in *C. benedictus* through new *in silico* techniques and predictive tools such as SwissADME and PubChem.

The data from the *in silico* study presented here support the traditional use of *C. benedictus*, as well as its promise as a source of new therapeutic chemical compounds.

Introduction

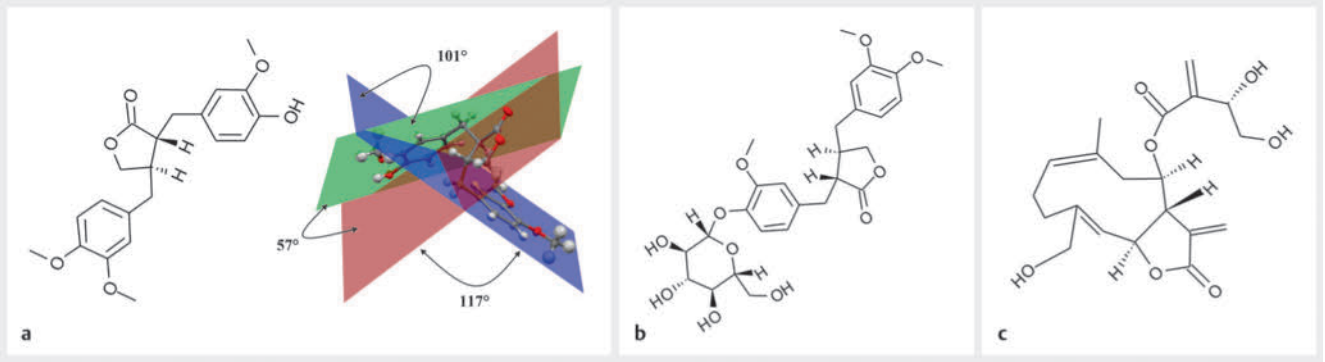
Blessed thistle (*Cnicus Benedictus* L., syn. *Centaurea benedicta* L.) is an annual plant belonging to the *Asteraceae* family. Originating from the Mediterranean region, it has been used in folk medicine for centuries [1].

The upper parts of the above-ground shoots constitute the medical raw material and are obtained during the plant's flowering period. On the pharmaceutical market, there is a range of products containing blessed thistle, including dietary supplements and medical preparations. Blessed thistle is primarily used to prepare infusions by steeping a small amount of the raw material in boiling water, as well as for the preparation of extracts and tinctures [2, 3]. This article reports broadly on the traditional use and pharmacological activity of *C. benedictus* and a computational analysis using novel methods to investigate the pharmacokinetic and toxicological profiles of the main active constituents cnicin, arctigenin, and arctiin–responsible for the pharmacological effects of this plant.

Methods

Our review of documented medicinal and known traditional uses of *C. benedictus* and its constituents is based on the literature available in the electronic databases PubMed, Toxline, and the Cochrane Library. Publications were filtered by using the following terms: *Cnicus benedictus, Centaurea benedicta*, blessed thistle, cnicin, arctigenin, and arctiin.

To investigate the pharmacokinetic and toxicological profiles of the main active phytochemicals of *C. benedictus*, publicly available tools such as PubChem, SwissADME, and Chem Mine Tools were used. SwissADME is a freely accessible web tool designed to



▶ **Fig. 1** Molecular structure, (**a**) arctigenin (2D semi-structural scheme and 3D stick representation with indicated planes of rings [8] (**b**) artiin; (**c**) cnicin (2D semi-structural scheme).

analyze the pharmacokinetics and drug-likeness of molecules based on *in silico* models and uses a variety of descriptors based on the rule-of-five (RO5) criteria compiled by Lipinski et al. (2001), such as, for example, molecular weight, logP, number of hydrogen bond donors and acceptors, and topological polar surface area (TPSA) [4, 5]. In order to qualify a compound as orally active and having suitable oral bioavailability, it has to fulfill the RO5 criteria. These criteria include the following characteristics: a molecular weight less than 500, a logP less than 5, hydrogen bond donors fewer than 5, and hydrogen bond acceptors fewer than 10. Rai et al. (2023) presented the assumptions that guided the *in silico* study, from inputting the compound to interpreting the results through the SwissADME suit [6]. The Tanimoto similarity coefficient (Tsc) is a commonly employed measure of molecular similarity for pairwise comparison of molecules. According to its description, this value ranges from 0.0 (indicating full dissimilarity) to 1.0 (indicating equal molecules) [7].

Chemical structure of the tested molecules

Only the structure of arctigenin QOFJIF01 is deposited in the Cambridge Structural Database (CSD) [8]. As we can see from ▶ **Fig. 1**, it is a molecule with a three-dimensional structure.

Arctigenin and artiin are phenylpropanoid dibenzylbutyrolactone lignans [9]. The lignan dibenzylbutyrolactones are phenylpropanoid metabolites from plants. They are phenolic compounds consisting of two phenylpropane units (C6-C3) linked by the side chain C-8, C-8′ carbon atoms [10, 11]. The structural analogs of arctigenin and artiin exhibit a Tanimoto coefficient of 0.71. Trachelogenin, a rare natural plant-derived lignan, exhibits the highest structural similarity to arctigenin, as indicated by a Tanimoto coefficient of 0.96. The antiviral, anti-inflammatory, and analgesic actions of trachologenin can be attributed to this structural similarity, as indicated by IC_{50} values of 0.325 and 0.259 µg/ml in the HCVcc and HCVpp models, respectively [12].

Cnicin is classified as a sesquiterpene lactone of the guaianolide group. Sesquiterpene lactones are terpenes that have a uniform structure consisting of 15 carbon atoms. This structure is formed through the biosynthesis of three isoprene units, and a five-membered lactone rings consists of hydroxycarboxylic acid cyclic esters. The lactone component exhibits a α-methylene-γ-lactone structure, which is a ring combining oxygen with a carbonyl group in the β position (O=C...C=CH2) [13]. They are colorless, durable, and hydrophobic substances. The sesquiterpene lactones have a wide range of biological actions. The lactone is thought to be the primary cause of the biological effects of sesquiterpene lactones on organisms and cells. A Michael-type addition reaction occurs between unsaturated carbonyl structures and nucleophiles in biological systems, as exemplified by the sulfhydryl group (SH) present in the amino acid cysteine. It is important to highlight the fact that more than 90% of human genome–encoded polypeptides contain the amino acid cysteine [14].

The active constituents of *C. benedictus*

C. benedictus contains a variety of chemical compounds, such as glycosides, triterpenoids, lignans, flavonoids, phenolic acids, steroid compounds, volatile oils (0.3%), tannins (8%), and mineral components (▶ **Table 1**) [1, 3, 15–21]. The most active, well-known substances responsible for the bitter taste and pharmacological effects of *C. benedictus* are the glycosides cnicin, arctigenin, and arctiin [22].

Biological activity and use of *C. benedictus* in traditional medicine

Most contemporary recommendations regarding the therapeutic use of *C. benedictus* are based on the established position of this plant in traditional medicine (▶ **Table 2**). There is a lack of controlled clinical studies supporting the beneficial effects of preparations containing *C. benedictus* in humans.

In folk medicine, an infusion made from *C. benedictus* is considered a general tonic, expectorant, and beneficial for the digestive system [3]. Despite a limited amount of high-quality scientific data, the bitter components in *C. benedictus* make it traditionally used for gastrointestinal problems. It is believed to increase the secretion of gastric juice and saliva, counteract excessive intestinal fermentation, reduce bloating and indigestion, improve appetite, and influence liver function by promoting bile production and secretion [2, 23–25]. According to the Committee on Herbal Medicinal Products of the European Medicines Agency, *C. benedictus* products are used traditionally for temporary loss of appetite and

▶ **Table 1** The active constituents of *C. benedictus* L.

sesquiterpene lactone	cnicin artemisiifolin absyntina salonitenolid cnicynolid melitensin, melitensin 15-O-β-D-glucoside 11β,13-dihydrosalonitenolide 15-O-β-D-glucoside parthenolide
lignans	arctigenin trachelogenin nortracheloside
triterpenoids	α-amyrenon, α-amyrenon acetate α-amyrine, α-amyrin acetate multiflorenol acetate
flavonoids	luteolin apigenin-7-O-glucoside astragalin
steroids	phytosterol stigmasterol multiflorenol
phenolic acids	chlorogenic acid sinapic acid
volatile oils	citral p-cymene fenchon cinnamaldehyde
mineral contents	magnesium manganese calcium potassium sodium phosphorus

▶ **Table 2** The use of *C. benedictus* L. in traditional medicine (based on available literature).

internal	digestive disorders, indigestion, dyspepsia, diarrhea lack of appetite, anorexia bloating cervical dysplasia menstrual irregularities lactation stimulant reconvalescence infectious diseases
external	wounds ulcers swelling

symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract [26].

One of the plant's active components, cnicin, has demonstrated hepatoprotective properties, making it potentially valuable in the management of liver diseases. Both the British and German pharmacopoeias state that the active ingredients in *C. benedictus* act as cholagogues [27]. In the past, large doses were used as emetics, and the plant's seeds were employed as an emmenagogue.

It is also considered to be a galactagogue, meaning a substance that increases and stimulates lactation in women [2, 28], and an effective analgesic for menstrual pain [1]. In 2019, a questionnaire survey was conducted in Australia among breastfeeding mothers who used *C. benedictus* preparations to stimulate lactation. The majority of women participating in the study rated the effectiveness of *C. benedictus* as a lactogenic agent as low or moderate [29]. However, it should be emphasized that due to the lack of sufficient data from clinical trials, the product is not recommended by the European Medicines Agency for pregnant and lactating women [26].

The active ingredients present in the plant regulate metabolism and exhibit antibacterial and anti-inflammatory properties. Despite limited evidence for its effectiveness, the herbal remedy is traditionally used as a diuretic [30, 31].

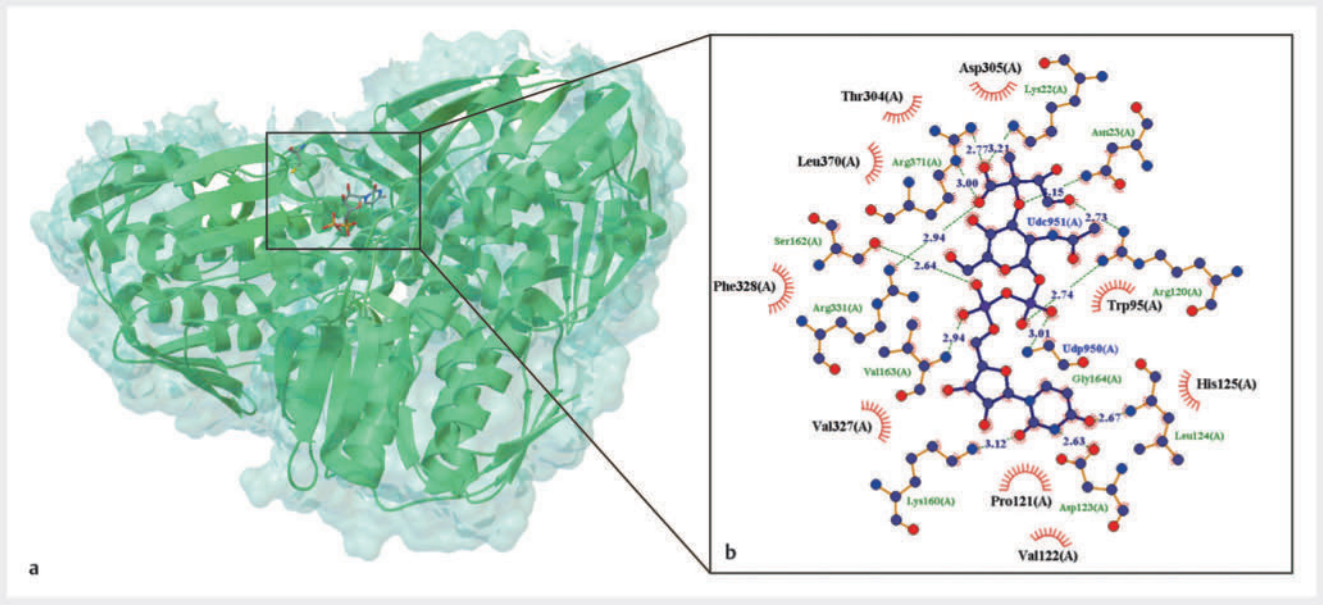
Traditionally, an infusion of *C. benedictus* was used to treat infectious diseases such as tuberculosis, malaria, and plague [32]. The herb was also used topically for dressing wounds and ulcers [31, 33]. Due to its high mineral content, *C. benedictus* was used during reconvalescence, and the infusion is still used in South Africa in the context of cancer. However, the effectiveness of such applications has not been adequately confirmed in clinical trials [2, 27, 34].

Antimicrobial, antiviral, and antiparasitic activity

The antimicrobial activity of the plant has been demonstrated against Gram-positive cocci and Gram-negative bacillus. Vanhaelen-Fastré (1973) investigated the antibacterial properties of the essential oil obtained from the herb of *C. benedictus* and showed that 15 out of 30 chromatographically identified substances, including p-cymene, fenchone, citral, and citronellol, exhibited such properties [35]. The main contributors to the antibacterial activity of the plant are sesquiterpene lactones, primarily cynarin [2, 36–38]. Antibiotic properties are also exhibited by essential oils, aromatic aldehydes (benzaldehyde, cinnamaldehyde, and cuminaldehyde), and monoterpenes (citronellol, fenchone, and p-cymene) [27]. However, it should be noted that the antibacterial properties depend on the type of extract tested and the origin of the plant raw material [16].

The extract of *C. benedictus* inhibited the growth of cultures of *Bacillus subtilis, Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Brucela species*, and *Enterococcus faecalis* [39, 40]. Yasin and Ibrahem (2017) demonstrated the antibacterial activity of the ethanolic extracts of the roots of this plant against bacteria such as *Escherichia coli, Bacillus pumillus, Staphylococcus aureus*, and *Micrococcus*. Additionally, the results of chemical analyses confirmed the presence of various active compounds in the roots of *C. benedictus*, including alkaloids, flavonoids, phenols, tannins, and terpenes. At the same time, no antibacterial activity was observed against the *Klebsiella* and *Pseudomonas* species, nor against *S. aureus, S. typhi*, and yeast [2, 40].

These properties were also confirmed by studies conducted in animal models. In rats, the effect of the powdered root was inves-



▶ **Fig. 2** Crystal structure of MURA inhibited by unag-cnicin adduct (**a**) with the enlarged area showing the structural elements around the ligand-biding site (PDB ID: 2Z2C, 2.05 Å) [45]. Residues that form hydrogen bonds (dashed lines) with cnicin are shown in ball-and-stick representation with the interatomic distances shown in Å. Residues forming Van der Waals interactions with UDP are shown as labeled arcs with radial spokes that point toward the ligand atoms (**b**).

tigated on the rate of healing of wounds that were experimentally induced by cutting a skin fragment on the back. After two weeks of application, the area of the wounds that had healed reached 95 %, confirming the effectiveness of the *C. benedictus* formulation (a homogeneous mixture of 10 g of the plant root powder with 50 g vegetable jelly "vaseline" of unknown composition). The impact of this plant preparation on the rate of healing of the experimentally induced wound was comparable to the effectiveness of the antibiotic ointment Baneocin, a composite preparation containing the antibiotics bacitracin and neomycin, which are used topically to treat bacterial infections [31].

In particular, cnicin exhibits significant antimicrobial activity against various pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria and fungi. Cnicin's antimicrobial action is mediated through the disruption of microbial cell membranes, inhibition of essential enzymes, and interference with microbial biofilm formation. Cell wall enzymes have consistently been a favored focus in drug exploration, owing to their crucial role in the survival of pathogens. According to Raina et al. (2021), the cytosolic enzyme MurA, also known as UDP-N-acetylglucosamine enolpyruvyl transferase (EC 2.5.1.7) [41], is essential for initiating the primary phase of bacterial cell wall production and has been identified as a target of numerous antibiotics. It has been shown that cnicin is a potential irreversible inhibitor of the bacterial enzyme MurA, which is crucial for bacterial survival and responsible for the initial step of the cytoplasmic biosynthesis of the precursor molecules of peptidoglycan [42]. The antibacterial properties of cnicin are likely conditioned by the presence of α, β-unsaturated carbonyl groups within the molecular chain. Thanks to these groups, cnicin forms stable bonds with the MurA enzyme through the thiol group of Cys115, ultimately leading to the alkylation of this important site and the complete blocking of bacterial enzyme activity [43]. The X-ray crystallographic investigations carried out by Skarzynski et al. in 1996 revealed that this spherical protein is composed of two different domains, each containing an equal number of α helices and β sheets [44]. These domains are interconnected by a double-stranded linker. The catalytic domain consists of residues 22–229; the catalytic site is located at Cys-115, which participates in product formation (▶ **Fig. 2a**) [44, 45]. The other domain, known as the C-terminal domain, consists of residues 1–21 and 230–419. The catalytic site is positioned in the cavity formed by these domains. ▶ **Fig. 2b** illustrates the hydrolysis of the ester function of cnicin, leading to the elimination of its macrocyclic component. The aforementioned mechanism is essential for the Cys115 loop to adopt the conformation evidenced in the MURA-fosfomycin complex [45]. Cnicin may also exert antibacterial effects through the formation of (−)-tulipalin B [46]. A study was conducted to evaluate the inhibitory effects of cnicin on the growth of *B. subtilis, S. epidermidis, E. coli, K. pneumoniae, P. aeruginosa*, and *S. aureus*. For *S. aureus* 25 923, 25 178, 6538, *E. coli, K. pneumonia*, and *P. aeruginosa*, the minimum inhibitory concentration (MIC) of the chloroform extract of cnicin is 124 µg/mL [1, 36, 47].

Barrero and coworkers (2000) evaluated the microbiological activity of sesquiterpene lactones against the filamentous fungus *Cunninghamella echinulata*. Upon incubation of *C. echinulata* with cnicin, hydrolysis of the side chain of this compound occurred, resulting in the formation of salonitenolide. No hydrogenation products or inhibition of fungal growth was observed. The greater polarity of sesquiterpene lactones is responsible for the lower antifungal activity. This may be related to the degree of lipophilic-

ity required for sesquiterpenoids to penetrate the fungal cell wall [47, 48].

Some data indicate the antiviral (anti-HIV) activity of lignans found in the herb of *C. benedictus*. There is also evidence of synergistic antiviral action between arctigenin, an important active compound in the plant, and oseltamivir (neuraminidase inhibitor) against oseltamivir-resistant H1N1 influenza viruses. The presence of arctigenin likely inhibits virus replication in host cells [49, 50]. The latest research demonstrates that arctigenin found in other plant species such as *Forsythia viridissima* fruit ethanol extract inhibits the replication of human coronavirus by suppressing viral protein expression and cytotoxicity [51].

C. benedictus is also considered a promising natural remedy in combating the SARS-CoV-2 virus, which, especially due to its mutations and the emergence of new variants, has been a global epidemic threat since 2019. In light of the danger of a resurgence of the pandemic, testing has begun to assess the efficacy of plant extracts, which are easily accessible sources for developing new antiviral drugs. Alhadrami et al. (2021) conducted *in silico* and *in vitro* experiments to investigate the potential of cnicin in inhibiting the replication of the SARS-CoV-2 virus. The results indicated that the inhibitory effect of cnicin was dose-dependent, with an IC_{50} value of 1.18 µg/ml [52], providing the impetus for conducting *in vivo* studies and clinical trials.

Queiroz and coworkers (2021) evaluated the anti-parasitic effect of cnicin on trematodes of the genus *Schistosoma*. Both *in vitro* and *in vivo* studies were conducted to assess the effectiveness of cnicin and its complexes with *β*-cyclodextrin (*β*CD) and 2-hydroxypropyl-*β*-cyclodextrin (HP*β*CD) against *Schistosoma mansoni*. The research findings showed that intraperitoneal administration of cnicin in mice caused changes in the covering of adult forms and led to the death of the parasite. In a murine schistosomiasis model, it was found that oral administration of cnicin or its *β*CD complex was ineffective and did not result in a significant reduction in the number of parasites. In contrast, high anti-parasitic efficacy against *S. mansoni* was demonstrated in mice when cnicin complexes with HP*β*CD were administered orally or intraperitoneally. These complexes reduced the number of adult parasitic forms and significantly limited the number of produced eggs. Permeability studies using a fluorescent dye indicated that the HP*β*CD complex could penetrate the covering of adult *S. mansoni* forms *in vivo* [53].

Despite the promising results of preclinical studies, the antimicrobial, antiviral, and antiparasitic properties of *C. benedictus* have not yet been sufficiently confirmed in clinical conditions.

Antioxidant, anti-inflammatory, and analgesic activity and neuroprotective benefits

In folk medicine, the herb from *C. benedictus* was used to treat pain, including painful menstruation [2]. In Algeria, the plant is a well-known remedy used for the treatment of wounds and burns.

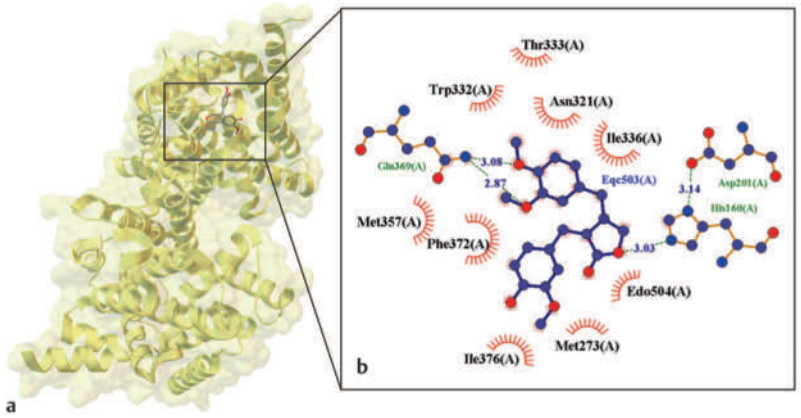
Many studies conducted on animals indicate anti-inflammatory and associated analgesic properties of the active ingredients of *C. benedictus*; however, such research has not been carried out in humans. Tests were conducted on mice to assess the impact of the plant extract and cnicin on the intensity of pain induced by formalin injection. Both a methanol extract of the leaves of *C. benedictus* and cnicin itself demonstrated antinociceptive effects, which could involve various neuronal signaling pathways and modulations, including the pathway associated with the L-arginine/nitric oxide/cGMP/ATP-sensitive potassium channel (LNCaP) and the endogenous opioid system [54].

In a preclinical study involving mice, the anti-inflammatory effects of cnicin and extracts from *C. benedictus* were demonstrated in models of inflammatory skin diseases. The animals were divided into groups, and solutions of various compounds inducing local inflammation were applied to ear skin. The inflammatory agents included croton oil, phenol, capsaicin, arachidonic acid, and histamine. Tissue levels of inflammatory markers, swelling size, leukocyte levels, and vascularization extent were then assessed in the collected ear fragments. It was observed that both the application of cnicin and *C. benedictus* extract (leaves extracted with dichloromethane : ethanol 9 : 1 v/v) led to a reduction in swelling and levels of inflammatory markers such as myeloperoxidase, N-acetyl-*β*-D-glucosaminidase, nitric oxide, tumor necrosis factor *α* (TNF-*α*), and interleukin-6 (IL-6). The study showed that cnicin interacts with cyclooxygenase-1 and nitric oxide synthase through hydrogen bonding, leading to inhibition of these key inflammatory enzymes [55].

Demiroz and coworkers (2018) evaluated the effect of cnicin in a rat model of paw inflammation induced by the injection of venom from Ottoman vipers of the species *Montivipera xanthina* and *Macrovipera lebetina obtusa*. The venom of these snakes induces tissue swelling, leading to necrosis of the skin and muscles. Cnicin was administered orally at three different single doses (2.5, 5, and 10 mg/kg b. w.), and its effectiveness was compared with indomethacin, a nonsteroidal anti-inflammatory drug. The area affected by snake venom–induced inflammation was extensive in the first hours after administration and decreased in the following hours. The study demonstrated that the anti-inflammatory effect of cnicin, assessed half an hour after the onset of swelling, was comparable to the effectiveness of indomethacin. The most effective dose was considered to be 10 mg/kg b. w. [56].

An *in vitro* study demonstrated that cnicin also has a neuroprotective effect, effectively promotes axon regeneration, and accelerates functional recovery after nerve injury [57].

Published data suggest that lignans such as arctigenin and trachelogenin can reduce cyclic adenosine monophosphate (cAMP) levels and inhibit phosphodiesterase (PDE) activity and mast cell histamine release [2]. The recognition of drug discovery targeting PDE4 as a competitive and promising technique for the development of anti-inflammatory medicines has gained significant attention in recent decades [58, 59]. Patients with psoriasis, psoriatic arthritis, atopic dermatitis, inflammatory bowel disease, and asthma exhibit a notable increase in the activity and distribution of PDE4 in peripheral immune cells and skin biopsies. The available evidence suggests that inhibition of PDE4 leads to a notable elevation in cAMP concentration, which subsequently triggers the activation of protein kinase A (PKA). The regulation of inflammation and tissue homeostasis was found to be concurrently influenced by the phosphorylation of nuclear transcription factors, including cAMP-responsive element binding protein (CREB), cAMP-responsive element modulator (CREM), and activated transcription factor 1 (ATF-1) [60].



▶ **Fig. 3** Crystal structure of PDE4D catalytic domain in complex with arctigenin (**a**) with the enlarged area showing the structural elements around the ligand-biding site (PDB ID: RLRM, 1.45 Å) [60]. Residues that form hydrogen bonds (dashed lines) with arctigenin are shown in ball-and-stick representation with the interatomic distances shown in Å. Residues forming Van der Waals interactions with arctigenin are shown as labeled arcs with radial spokes that point toward the ligand atoms (**b**).

Arctigenin has the ability to decrease the phosphorylation of Akt and PDE, resulting in a buildup of cAMP within cells and the activation of PKA. Crystallographic investigations have unveiled robust connections between arctigenin and the binding pocket of PDE4D, as depicted in ▶ **Fig. 3**. This finding provides evidence for the molecular-level inhibition of PDE4D by arctigenin. The experiments verified a moderate level of inhibitory action against PDE4D, as indicated by an IC50 value of 3.76 ± 0.28 µM [60].

Antagonistic effects of cnicin on calcium ions and platelet-activating factor (PAF) have also been observed in mast cells [61, 62].

The antioxidant effect of extracts from *C. benedictus* was confirmed in liver cells under conditions of oxidative stress. Oxidative stress was measured on the basis of the level of lipid peroxidation (LPO), glutathione content, total protein level, and enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) in the liver tissue homogenate. A significant improvement in most of the investigated variables was observed after the administration of the hydroethanolic extracts [63].

Arctigenin also exhibits anti-inflammatory and analgesic effects. This lignan demonstrates neuroprotective effects *in vitro* by reducing neurotoxicity induced by glutamate and kainic acid. Arctigenin inhibits the binding of kainic acid to many receptors, including glutamate receptors such as NMDA receptors, AMPA receptors, kainate receptors, and metabotropic glutamate receptors. Furthermore, there is empirical evidence for its efficacy in removing reactive oxygen species [64]. The mechanism of the antioxidant and anti-inflammatory action of arctigenin is complex and involves inhibiting the production and release of inflammatory mediators, such as arachidonic acid metabolites and free radicals. This leads to reduction in exudate formation and the inhibition of leukocyte migration into inflamed tissues [65]. *In vitro*, arctigenin also limits the production of nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) stimulated by lipopolysaccharide (LPS) in macrophage cell lines RAW 264.7 and THP-1. Arctigenin inhibits the expression and enzymatic activity of induced nitric oxide synthase (iNOS) caused by phorbol myristate acetate (PMA) and LPS [66, 67]. Additionally, it inhibits the phosphorylation of MAP kinases ERK1/2, p38 kinase, and JNK, and blocks the production of TNF-α [9, 68]. These anti-inflammatory effects make cnicin a promising candidate for the treatment of inflammatory diseases.

The neuroprotective benefits of arctigenin have been observed both *in vitro* and *in vivo*, specifically in models of Parkinson's and Alzheimer's disease. One of the pathological hallmarks of Alzheimer's disease is elevated phosphorylation and aggregation of tau, a neuronal microtubule-associated protein. In the hippocampus, arctigenin effectively reduced the phosphorylation of tau at Thr-181, Thr-231, and Ser-404. Additionally, arctigenin was found to enhance the phosphorylation levels of phosphatidylinositol 3-kinase, threonine/serine protein kinase B, and glycogen synthase-3-kinase β. Arctigenin has been found to be efficacious in safeguarding against cognitive impairments related to learning and memory, while also suppressing the development of hyperphosphorylated tau protein inside the hippocampus. In 2017, Qi et al. demonstrated that arctigenin treatment decreases tau hyperphosphorylation within the GSK-3β/PI3K/Akt- and PI3K/Akt/GSK-3β-dependent signaling pathways [69, 70].

Anticancer activity

In South Africa, infusions made from the herb of *C. benedictus* are used to treat cancer-related health problems; however, their effectiveness has not been confirmed by clinical studies. *C. benedictus* is also one of multiple ingredients in the Flor-Essence preparation, which has clinically unconfirmed anticancer properties. In Canada, the preparation is used by patients with cancer, as well as by individuals suffering from other chronic disease [2, 27].

Preclinical studies suggest that certain compounds found in the herb, such as cnicin, arctigenin, or arctiin, may have anticancer properties.

Tumor cells in poorly vascularized, solid tumors are constantly or periodically exposed to unfavorable microenvironmental conditions, such as glucose deficiency or hypoxia. Glucose deficiency often activates the unfolded protein response (UPR) pathway, involving improperly folded proteins. This pathway increases the survival of cancer cells by inducing stress proteins. Arctigenin selectively inhibits the viability of tumor cells under glucose-deficient conditions. This compound inhibits the expression of UPR genes, such as PERK, ATF4, CHOP, and GRP78, leading to the initiation of apoptosis through the activation of caspases 9 and 3 [71]. Arctigenin at a concentration of 0.01 µg/ml has been identified as the primary compound exhibiting preferential cytotoxicity against nutrient-deprived cells. It inhibits the growth of pancreatic tumors, such as PANC-1, in mice, exhibiting preferred cytotoxicity under nutrient-deprived conditions [72].

Arctiin and arctigenin also demonstrated beneficial effects in skin tumors induced by 7,12-dimethylbenz[a]anthracene and 12-O-tetradecanoylphorbol-13-acetate in mice. In rodents, these lignans caused an over 50% reduction in the number of papillomas over a period of 20 weeks. Additionally, arctigenin exhibited strong antitumor activity in a two-stage carcinogenesis test for mouse lung tumors induced by N-nitroquinoline oxide as an initiator and glycerol as a promoter of tumorigenesis. Oral administration of arctigenin significantly reduced the incidence of lung tumors, with approximately 50% inhibition in the total number of lung tumors and over 40% reduction in the percentage of mice with tumors in the lung lobe [73].

Other studies indicate that arctiin has a protective effect in the carcinogenesis induced by PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), especially within the mammary gland and colon. This is likely associated with the influence of this compound on the arachidonic acid metabolism pathway [74].

Studies have also highlighted the potential of cnicin as an anticancer agent. It exhibits cytotoxicity toward various cancer cell lines, including breast, lung, colon, and prostate cancers. Cnicin induces programmed cell death, inhibits cell proliferation, and modulates signaling pathways involved in cancer progression. Additionally, it has shown promising results in inhibiting angiogenesis, a process critical for tumor growth and metastasis. As part of the study on the anti-inflammatory properties of cnicin, the main active substance in *C. benedictus*, this compound was isolated using column chromatography, characterized through nuclear magnetic resonance (NMR) spectroscopy, and then subjected to cell tests to assess its inhibitory effect on nuclear factor κB (NF-κB), inducible nitric oxide synthase (iNOS) activity, generation of reactive oxygen species, and cytotoxicity in selected human solid tumor cell lines and in two non-tumor kidney cell lines (Vero monkey kidney fibroblasts and LLC-PK11 pig kidney epithelial cells). The results demonstrated inhibition of NF-κB and iNOS activity by cnicin. Cytotoxic effects were observed on LLC-PK11 pig kidney epithelial cells, human malignant melanoma cells (SK-MEL), and human breast adenocarcinoma cells (BT-549). Cnicin was not toxic to Vero cells but exhibited mild toxicity to LLC-PK11 cells [68]. Cnicin also showed fairly strong activity against a human breast carcinoma line (MCF-7), with an IC_{50} value of 3.25 µg/mL, possibly due to the a-methylene-c-lactone rings in its structure [75]. In addition, cnicin significantly inhibited NF-κB (1.8 µM) and SP-1 (16.0 µM) in human chondrosarcoma cells (SW1353) induced with phorbol myristate acetate and also inhibited iNOS activity (6.5 µM) in mouse macrophages (RAW264.7 cells) induced with lipopolysaccharide [68].

Jöhrer and colleagues (2012) conducted research evaluating the potential cytotoxic effects of cnicin on multiple myeloma cells, which belong to one of the most common hematologic malignancies. The study demonstrated a strong antiproliferative effect of this compound and its ability to induce cell death in cell lines and primary multiple myeloma cells, even in the presence of cytokines that support survival and provide a suitable microenvironment for the tumor. Activation of caspases, accumulation of reactive oxygen species, and reduction in NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) levels contribute to the cytotoxic action of cnicin. Microarray analysis to detect genes involved in cnicin-induced cell death highlighted the pro-oncogene *PIM2*. The Pim-2 protein is a novel kinase that conditions the survival of multiple myeloma cells *in vitro* and is strongly expressed in malignant cells but not in normal plasma cells *in vivo*. The results established that cnicin induces multiple modes of cell death in multiple myeloma, suggesting Pim-2 as a new therapeutic target [76].

In 1997, Barrero and colleagues isolated malacytanolid, a product of the epoxidation and cyclization of cnicin. The cytotoxic activity of this compound was investigated *in vitro* in the mouse lymphoma cell line P-388, SCHABEL, and three human cell lines: A-549 (lung cancer), HT-29 (colon cancer), and MEL-28 (melanoma). The results indicate that this compound is 20–40-fold more cytotoxic than cnicin toward the P-388, A-549, and HT-29 cell lines [77].

It has been demonstrated that arctiin inhibits the growth of cancer cells by downregulating the expression of cyclin D1 and upregulating genes for MUC-1 (anti-adhesion mucin). Cyclin D1 is responsible for cell cycle regulation, and its overexpression is observed in many types of human cancers, such as breast cancer, prostate cancer, kidney cancer, and colorectal cancer. *In vitro*, arctiin inhibits the growth of PC-3 (human prostate cancer) and HaCaT (human immortalized keratinocyte) cell lines [78]. Arctigenin has been shown to block the activity of enzymes with tyrosine kinase activity (Akt) induced by glucose deficiency. Akt plays a crucial role in developing tolerance to starvation, increasing the survival of cancer cells under glucose deficiency conditions. Incubating cancer cells with arctigenin abolished the tolerance of cancer cells to nutrient-deficient conditions [72]. Inhibitory effects of arctigenin have also been demonstrated in the human promyelocytic leukemia HL-60 cell line, likely through blocking DNA, RNA, and/or protein synthesis in leukemia cells [79].

In *in vitro* studies, it has been demonstrated that cnicin, arctigenin, and arctiin inhibit the proliferation of DU-145 prostate cancer cells, MCF-7 (breast cancer cells), and MCF-12A (non-malignant breast cells). In contrast, an extract from *C. benedictus* does not exhibit such activity. This is an interesting discovery that highlights the differences in anticancer activity between individual isolated active compounds and a whole-plant extract, which is a mixture of various pharmacologically active compounds [34].

Despite promising research results, the anticancer properties of individual components of the plant should be confirmed in clinical conditions.

Safety profile of *C. benedictus*

Side effects

The published data so far regarding the safety of using *C. benedictus* indicate its low toxicity, provided that proper dosages are observed, with values based on traditional medicine practices. The plant is used orally as an herbal tea or as herbal preparations in liquid or solid dosage forms [26]. There is not enough reliable information to definitively confirm whether preparations of *C. benedictus* are safe as medicines and what adverse effects they may induce. Consuming an infusion or decoction of the herb in large quantities (> 5 g per cup) may lead to gastric irritation, nausea, vomiting, colic, diarrhea, and hypertension. Allergic reactions to components present in the herb, especially in individuals sensitive to plants from the *Asteraceae/Compositae* family, have also been observed [26, 80].

Laboratory studies suggest that *C. benedictus* may increase the risk of bleeding; however, such an effect has not been observed in humans. The herb contains tannins (approximately 8%), which theoretically may exhibit hepatotoxic and nephrotoxic effects with prolonged use [2]. Prolonged use of plant materials containing tannins in amounts exceeding 10% has been shown to cause gastrointestinal discomfort, liver disease, and kidney damage and increase the risk of esophageal or nasal cavity cancer [1, 26]. For this reason, caution should be exercised in patients with gastrointestinal ulcers, reflux disease, hiatal hernia, and Barrett's esophagus.

In traditional medicine, *C. benedictus* is used to stimulate milk secretion. However, it should be emphasized that the available literature lacks sufficient data on the penetration of active ingredients from the plant into breast milk and any potential impact on the health of breastfed infants. In a randomized, double-blind clinical trial, the effectiveness of a specific tea called Mother's Milk Tea (Traditional Medicinals, Sebastopol, CA) was compared with lemon verbena tea in mothers experiencing breastfeeding issues. Each tea bag contained 35 mg of *C. benedictus*, along with several other herbs. Mothers were advised to drink 3 to 5 cups of tea per day. No differences were observed between the groups regarding adverse symptoms in mothers and infants related to the digestive, respiratory, or dermatological systems, etc. There were also no noted differences in the growth parameters of breastfed infants between the two groups [81].

To this day, there have been no reports of teratogenic effects of *C. benedictus* used in pregnant women. However, the lack of preclinical studies in animals and controlled clinical trials in humans does not rule out potential harm to the fetus, especially since, in traditional medicine, the plant is used as an abortifacient and menstruation-inducing agent [2]. For this reason, its use during pregnancy and lactation is not recommended [26].

Simultaneously, in the absence of sufficient data, the use of *C. benedictus* in children and adolescents under 18 years of age has not been established and is not recommended [26].

Interactions with other medications

There is a lack of confirmed clinical data in the available literature regarding the interactions of preparations containing *C. benedictus* with other medications. The herb is traditionally used to increase gastric secretion, and there is a risk of interactions between the active ingredients of this plant and antacids used in peptic ulcer disease. Laboratory studies suggest that the use of preparations containing *C. benedictus* may increase the risk of bleeding, especially when combined with anticoagulant medications such as warfarin, heparin, antiplatelet drugs, and nonsteroidal anti-inflammatory drugs [2]. *C. benedictus* contains components that act antagonistically to platelet-activating factor (PAF), which theoretically could reduce platelet aggregation stimulated by this factor and contribute to bleeding [1].

The lignans present in the plant exhibit antiviral activity. Synergistic antiviral activity has been demonstrated between arctigenin and oseltamivir, a neuraminidase inhibitor, against resistant influenza viruses (H1N1) [49, 50].

In silico analysis of the pharmacokinetic and toxicological profiles of the main active phytochemicals in *C. benedictus*

Safety pharmacology is a rapidly expanding field that focuses on evaluating the possible dangers of improper pharmacotherapy. Evaluating the safety of a chemical is a crucial aspect of introducing a new medicine to the market. The Organisation for Economic Co-operation and Development (OECD) has published several principles for conducting drug safety testing [82]. Contemporary technology provides a range of methods that, when utilized, should establish the circumstances necessary to implement safety pharmacology.

Without a doubt, *in silico* studies provide several opportunities to find answers in this field. Currently, the primary focus is on studying the pharmacokinetic and toxicological properties of potential medication candidates in order to better understand them. The *in silico* analysis described below contributes significantly to this understanding.

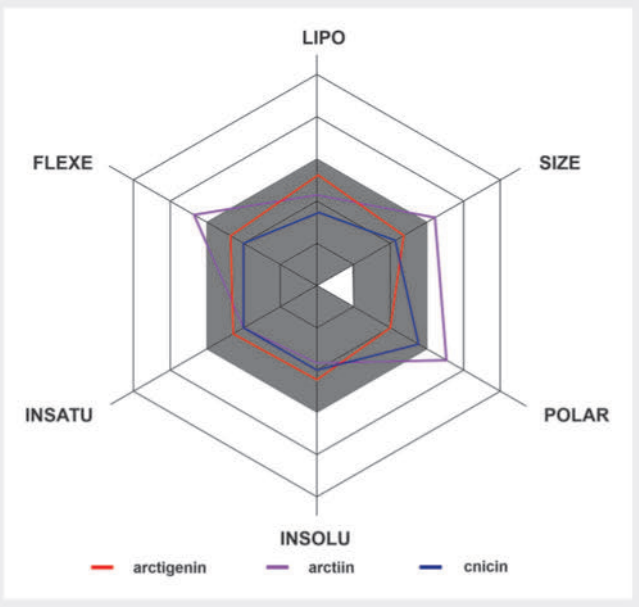
The bioavailability of cnicin, arctigenin, and arctiin

One of the goals of this study was to use SwissADME to forecast the physicochemical qualities, drug-likeness properties, ADME, and toxicity of three active constituents of *C. benedictus* to understand their pharmacokinetics and the traditional use of the plant.

The two-dimensional chemical structures of cnicin, arctigenin, and arctiin with canonical SMILES were extracted from Pubchem databases, and the bioavailability radar was constructed for each analyzed molecule (▶ **Fig. 4**). The bioavailability radar allows for the initial assessment of the drug-likeness of the tested molecules based on six physicochemical properties: lipophilicity (LIPO) measured by XLOGP3, which ranges from − 0.7 to + 5.0, size (SIZE) determined on the basis of molecular weight (MW) ranging from 150 to 500 g/mol, polarity (POLAR) resulting from the topological polar surface area (TPSA) spanning from 20 to 130 $Å^2$, insolubility (INSOLU), insaturation (INSATU), and flexibility (FLEX). Solubility is determined on the basis of log S, which should not exceed 6. In turn, insaturation is characterized on the basis of the fraction of carbons in the sp^3 hybridization (it should be no less than 0.25). Flexibility is assessed on the basis of the number of rotatable bonds (which should not exceed 9) [6, 83, 84]. It should be noted that the grey area designates the optimum physicochemical space for properties predicting bioavailability by oral administration.

▶ **Table 3** General characteristics of the phytoconstituents of *C. benedictus* L.

Small molecule	Pubchem ID	Molecular formula	Canonical SMILES	Molecular weight (g/mol)
cnicin	5281435	$C_{20}H_{26}O_7$	CC1=CCCC(=CC2C(C(C1)OC(=O)C(=C)C(CO)O)C(=C)C(=O)O2)CO	378.4
arctigenin	64981	$C_{21}H_{24}O_6$	COC1=C(C=C(C=C1)C[C@H]2COC(=O)[C@@H]2CC3=CC(=C(C=C3)O)OC)OC	372.4
arctiin	100528	$C_{27}H_{34}O_{11}$	COC1=C(C=C(C=C1)C[C@H]2COC(=O)[C@@H]2CC3=CC(=C(C=C3)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)OC)OC	534.6



▶ **Fig. 4** The bioavailability radar of the main components of C. benedictus L.: arctigenin, arctiin and cnicin. The gray area represents the optimal range for each of the properties (lipophilicity: XLOGP3 between − 0.7 and + 5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å2, solubility: log S no higher than 6, saturation: fraction of carbons in the sp3 hybridization no less than 0.25, and flexibility: no more than 9 rotatable bonds). The compounds names are indicated in the different font colors.

In ▶ **Tables 3–6**, we present the molecular and physicochemical characteristics of cnicin, arctigenin, and arctiin, including their molecular formulas, molecular weights (▶ **Table 3**), numbers of heavy atoms, numbers of aromatic heavy atoms, Csp^3 fractions, numbers of rotatable bonds, numbers of H-bond acceptors, numbers of H-bond donors, molar refractivities, TPSAs (▶ **Table 4**), lipophilicity characteristics (▶ **Table 5**), and water solubility (▶ **Table 6**).

Lipophilicity is demonstrated as partition coefficients (log P) and distribution coefficients (log D). Larger values of a small molecule's log P correspond to greater lipophilicity. To evaluate the lipophilic characteristics of *C. benedictus* compounds, we used five freely available models from SwissADME: XLOGP3 [85], WLOGP [86], MLOGP [87, 88], SILICOS-IT, and iLOGP [84].

Brain or intestinal estimated permeation method (BOILED-Egg) for blood–brain barrier (BBB) and gastrointestinal absorption prediction

To predict the brain penetration and gastrointestinal absorption of cnicin, arctigenin, and arctiin, we used the BOILED-Egg method [89–91]. The method was validated using a set of known compounds with experimentally determined permeabilities and involves estimating the partition coefficients of the tested molecules between an acetanol phase in the brain and intestines, which allows the calculation of predicted permeability across both the blood–brain barrier, and the gastrointestinal tract using the following equation:

1. Permeability across the blood–brain barrier (BBB)

$$\log(Pe_{brain}) = \log(P_{octanol}) - 0.67\log(P_{intestine}) - 0.0062 \quad (1)$$

Pe_{brain}: the predicted permeability coefficient across the BBB
$P_{octanol}$: the octanol–water partition coefficient
$P_{intestine}$: partition coefficient between intestinal tissue and octanol

2. Permeability across the gastrointestinal tract (GIT)

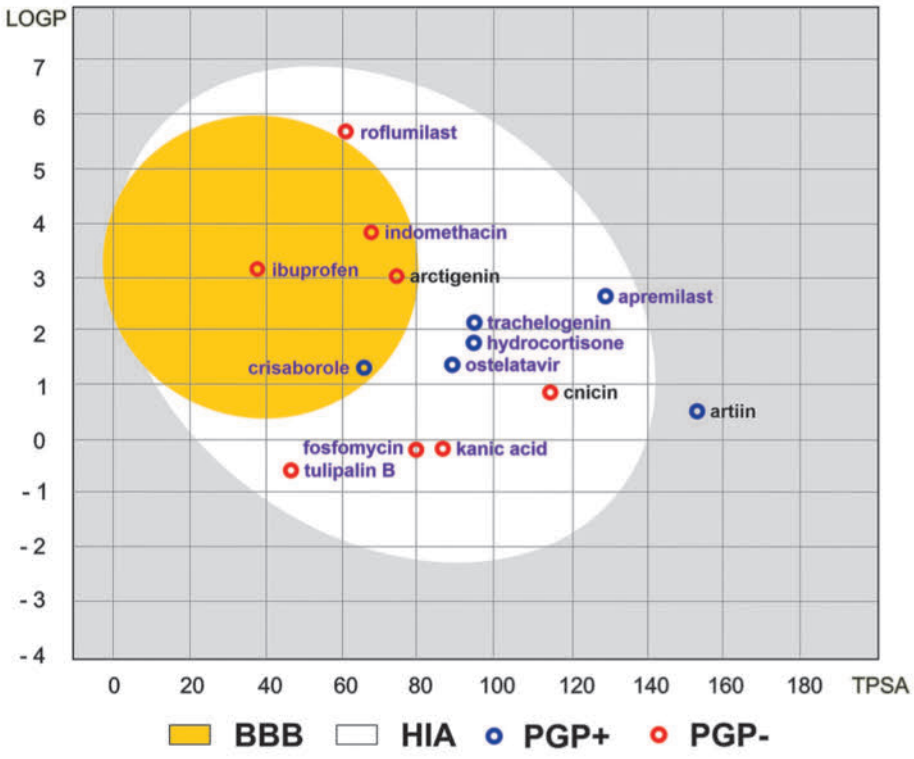
$$\log(Pe_{GIT}) = \log(P_{octanol}) + 0.43\log(P_{intestine}) - 0.34 \quad (2)$$

Pe_{GIT}: the predicted permeability coefficient across the GIT

The pharmacokinetic and drug-likeness assessment performed by SwissADME confirmed a high level of GI absorption with arctigenin and cnicin and high BBB permeation by arctigenin. Only arctiin present in *C. benedictus* L. is a substrate for P-gp (▶ **Fig. 5**).

More extensive SwissADME predictions of the pharmacokinetics and other properties of arctigenin, cnicin, and arctiin are presented in ▶ **Table 7**. Arctigenin is predicted with high probability to be an inhibitor of the cytochrome P 450 isoenzymes CYP2C9, CYP2D6, and CYP3A4. Almost all of the analyzed small molecules except arctiin returned as non-substrates of P-gp. Arctiin is also probably a CYP3A4 inhibitor.

This study predicts that only arctigenin will be found in the yolk in the assay, indicating a significant level of brain penetration. On the other hand, cnicin is expected to be present in the white zone of the BOILED egg, indicating a high level of passive gastrointestinal absorption. Conversely, arctiin has the lowest probability of being absorbed by the BBB and hematoma. The comparative analysis presented here demonstrates a significant pharmacokinetic resemblance to NSAIDs such as ibuprofen and indomethacin. Cnicin exhibits a bioactivity profile similar to that of kainic acid. Kainic



▶ **Fig. 5** Boiled-EGG model for analyzing gastrointestinal absorption (GI) and blood brain barrier (BBB) permeant P-gp substrate. White region is for high probability for passive absorption by GI and the yellow area (yolk) is for high probability of brain penetration. Points colored in blue is predicted as actively effluxes by P-gp (PGP+) and in red if predicted as non-substrate of P-gp (PGP-). The names of the compounds are indicated in different font colors, i.e. black corresponds to the analyses active compound, and purple corresponds to the selected drugs for comparison.

▶ **Table 4** Physicochemical properties the phytoconstituents of *C. benedictus* L. Num.–number, arom. -aromatic, H-bond–hydrogen bond, TPSA–topological polar surface area.

Molecule	Num. heavy atoms	Num. arom. heavy atoms	Fraction Csp3	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar refractivity	TPSA (0A2)
cnicin	27	0	0.50	6	7	3	98.19	113.29
arctigenin	27	12	0.38	7	6	1	100.60	74.22
arctiin	38	12	0.52	10	11	4	132.72	153.37

▶ **Table 5** Lipophilicity characteristics of the phytoconstituents of *C. benedictus* L.

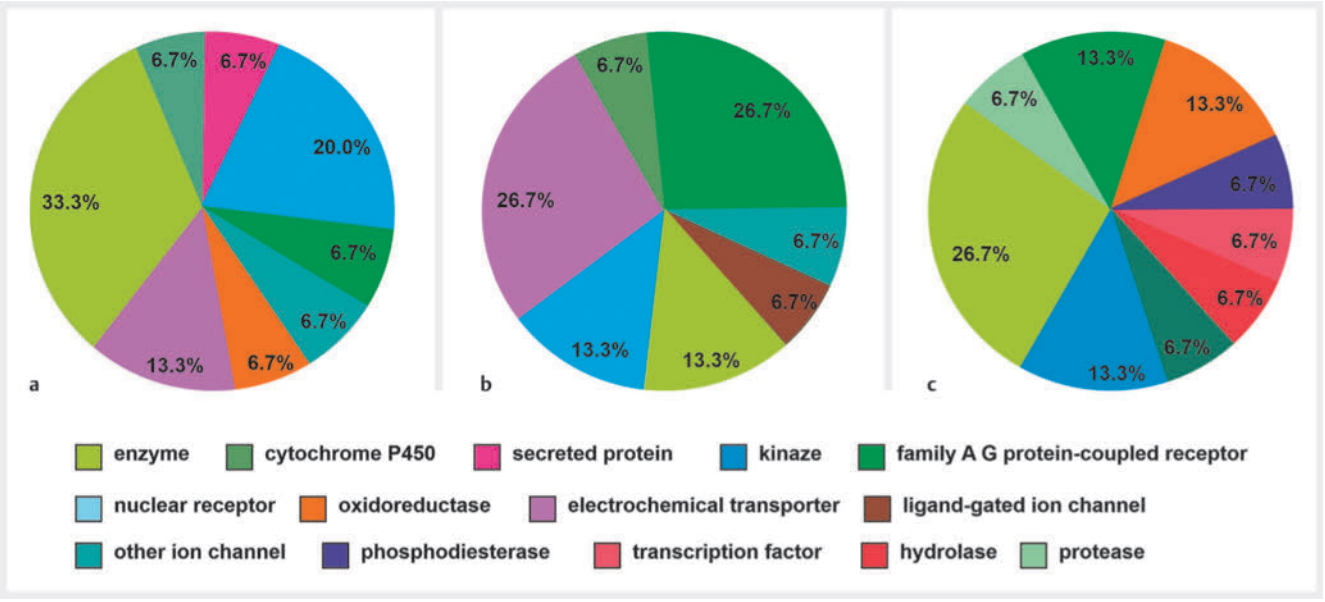
Molecule	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log Po/w
cnicin	1.97	0.25	0.95	0.92	1.82	1.18
arctigenin	2.64	3.59	2.99	2.12	3.99	3.07
arctiin	2.73	1.78	0.47	−0.11	1.93	1.36

▶ **Table 6** Water solubility characteristics of the phytoconstituents of *C. benedictus* L. S–soluble, MS–moderately soluble, VS–very soluble.

Molecule	ESOL				Ali				SILICOS- IT			
	Log S (ESOL)	Solubility		Class	Log S (Ali)	Solubility		Class	Log S (SILICOS- IT)	Solubility		Class
		mg/ml	mol/L			mg/ml	mol/L			mg/ml	mol/L	
cnicin	− 1.95	4.27	1.13×10^{-2}	VS	− 2.19	2.44	6.46×10^{-3}	S	− 1.64	8.75	2.31×10^{-2}	S
arctigenin	− 4.28	1.97×10^{-2}	5.28×10^{-5}	MS	− 4.84	5.44×10^{-3}	1.46×10^{-5}	MS	− 5.75	6.56×10^{-4}	1.76×10^{-6}	MS
arctiin	− 3.85	7.56×10^{-2}	1.41×10^{-4}	S	− 4.62	1.29×10^{-2}	2.40×10^{-5}	MS	− 3.99	5.51×10^{-2}	1.03×10^{-4}	S

▶ **Table 7** Pharmacokinetic parameters of the phytoconstituents of *C. benedictus* L.

Molecule	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (Skin Permeation) (cm/s)
cnicin	high	no	no	no	no	no	no	no	− 8.43
arctigenin	high	yes	no	no	no	yes	yes	yes	− 6.02
arctiin	low	no	yes	no	no	no	no	yes	− 8.30



▶ **Fig. 6** Target prediction for cnicin (**a**), arctigenin (**b**) and arctiin (**c**) cnicin made using SwissTargetPrediction..

acid can be classified as a neurotoxin when consumed in high amounts, whereas low amounts of a diluted solution can chemically excite neurons [92]. Arctigenin is a competitive inhibitor of kainic acid binding.

Target predictions for cnicin, arctigenin, and arctiin

We identified the most probable macromolecular targets of the tested molecules cnicin (▶ **Fig. 6a**), arctigenin (▶ **Fig. 6b**), and arctiin (▶ **Fig. 6c**) using the web tool SwissTargetPrediction. The identifications are based on a combination of 2D and 3D similarity with a library of 370 000 known activities on more than 3000 proteins from three different species: *Homo sapiens, Mus musculus*, and *Rattus norvegicus* [93, 94]. A combined score above 0.5 indicates that the small molecules share a common protein target.

The following comprised most of the arctigenin targets identified using the latest version of SwissTargetPrediction: cytochrome P450, oxidoreductase, family AG protein, nuclear receptor, and electrochemical transporter enzyme. Identified high-probability targets of arctigenin were secreted proteins such as sex hormone–binding globulin (SHBG), which is important for reproduction, and kinases such as mitogen-activated protein kinase kinase 1 (MAP2K1).

Cnicin was predicted to target the following molecular/biochemical pathways: phosphodiesterase, protease, cytochrome P450, oxidoreductase, enzyme, hydrolase, family AG protein, and transcription factor.

Arctiin targets included family AG protein, kinase, nuclear receptor, enzymes, electrochemical transporter, and ligand-gated ion channel.

Discussion

Data from preclinical *in vitro* and *in vivo* studies present an extensive picture of the pharmacological activity of the plant. We conclude that *C. benedictus* has multiple pharmacological activities and that this still-underestimated and little-known plant is a source of many unique active compounds of potential therapeutic importance. One of the pharmacologically active compounds is cnicin, a sesquiterpene lactone that possesses diverse pharmacological properties, including anti-inflammatory, anticancer, hepatoprotective, and antimicrobial activities. Multiple studies have demonstrated its ability to inhibit the production of pro-inflammatory mediators and prostaglandins. Studies have highlighted the potential of cnicin as an anticancer agent and its cytotoxic effects against various cancer cell lines, including breast, lung, colon, and prostate cancers. Cnicin induces programmed cell death, inhibits cell proliferation, and modulates signaling pathways involved in cancer progression. Additionally, it has shown promising results in inhibiting angiogenesis, a process critical for tumor growth and metastasis. *In silico* studies conducted to evaluate the antiviral efficacy of *C. benedictus* and its potential inhibitory impact on virus replication *in vitro* demonstrated that cnicin has a broad spectrum of antiviral activity while simultaneously exhibiting a favorable safety profile [52]. All currently available and well-characterized viral target proteins were used to determine the antiviral potential of the main active ingredients present in the plant extract, followed by *in vitro* activity assessments. The analysis revealed that cnicin interacts with nine targets involved in virus replication, host cell entry, and the cellular immune response to infection. Furthermore, it was found that cnicin dose-dependently inhibits the replication of the SARS-CoV-2 virus. This provides the basis for further *in vivo* research and clinical trials to evaluate the antiviral effectiveness of both the entire plant and its individual active components [52].

This study is the first to report the ADME (absorption, distribution, metabolism, and excretion) characteristics of *C. benedictus* components using the freely available web tool SwissADME. The plant's main reported active compounds were screened for ADME properties, and the resulting BOILED-Egg representation revealed that arctigenin has the ability to penetrate the brain and may be passively absorbed from the gastrointestinal tract. Arctigenin exhibits remarkable biological activity, making it a promising candidate for further advancement as a lead compound. Given the known pharmacological actions of arctigenin, there has been a growing trend toward modifying its structure. Arctigenin's inadequate efficacy, poor solubility, and constrained bioavailability are its limitations. The structure of arctigenin has been modified mostly to make it more biologically active, more soluble, and more amenable to drug development [95]. Our *in silico* analysis predicted that MAP2K1 is an important arctigenin target. MAP2K1 acts as an integration point for multiple biochemical signals and is involved in cellular processes including proliferation, differentiation, transcriptional regulation, and development. Activating mutations in MAP2K1 are involved in a variety of cancers, and MAP2K1 is a potential therapeutic target for anticancer drugs [96].

Cnicin also has properties that allow for good gastrointestinal absorption. This is confirmed by research conducted by Gobrecht et al. (2024), who suggest that repeated oral administration of cnicin (800 µg) in rats fully mimics the effects observed after intravenous cnicin; however, peak plasma concentrations were approximately 6 times lower. Analysis of cnicin pharmacokinetics after a systemic dose of 800 µg shows a blood half-life of 12.7 min, with high absolute oral bioavailability that reaches 84.7 % [57].

Based on the analysis carried out using SwissADME, it can be assumed that arctiin is not absorbed from the gastrointestinal tract, but its metabolites are very likely to be. This has been confirmed in the literature. Preclinical studies in animals indicate that after oral administration of the herb, lignans such as arctiin and tracheloside are metabolized to the genins arctigenin and trachelogenin. The maximum blood concentration of arctigenin is observed after 4 hours and of trachelogenin after 8 hours. These experiments suggest that during the metabolism of lignans in the gastrointestinal tract, the glycosidic bond is first cleaved, and then the phenolic methoxy group is demethylated [2, 61].

The presented *in silico* studies of the active ingredients of *C. benedictus* are valuable due to the fact that the available literature lacks data on the pharmacokinetics of the active compounds of this plant in humans. The literature mentions only the pharmacokinetics of some active compounds found in the plant. Research carried out in 2003 showed that anaerobic incubation of arctiine, one of the compounds present in the herb, with a suspension of human feces leads to the formation of metabolites with estrogenic and antiestrogenic properties [97].

Numerous *in vitro* and *in vivo* studies have demonstrated that the health-promoting effects of phytochemical compounds contained in the *C. benedictus* plant result mainly from their antioxidant potential. Rezig and coworkers (2024) conducted a molecular docking evaluation of six phenolic compounds of *C. benedictus* root extract against enzymes engaged in inflammation, as well as enzymes linked to Alzheimer's disease. Binding energies and analyses of molecular interactions revealed that the flavone and 3-hydroxy flavone moieties have good binding energy after docking with cyclooxygenase 2, acetylcholine esterase, and butyl choline esterase [16].

The *in silico* studies presented here demonstrate that the active ingredients found in *C. benedictus* have the potential to interact with many molecular targets, including kinases, other enzymes, and transport proteins. The *in vivo* activity is the result of many factors that modulate reactivity or physicochemical properties, among which the chemical structure is the most important. The data from the study support the traditional use of *C. benedictus* as a health-promoting herb. The compounds found in the plant show great promise in natural product–based drug development. However, further research, including preclinical and clinical studies, is needed to fully understand the therapeutic potential of compounds derived from *C. benedictus*, optimize their dosage regimens, and assess their safety profiles.

Contributors' Statement

Data collection: K. Zietal, K. Blecharz-Klin, A. Nowaczyk; design of the study: K. Zietal, K. Blecharz-Klin; analysis and interpretation of the data: Blecharz-Klin, A. Nowaczyk; drafting the manuscript: K. Zietal, K. Blecharz-Klin, A. Nowaczyk; critical revision of the manuscript: D. Mirowska-Guzel

Funding Information

The authors declare no competing interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The project was implemented with CePT infrastructure financed by the EU: the European Regional Development Fund within the Operational Programme "Innovative economy" for 2007–2013.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Tiwana G, Fua J, Lu L, Cheesman MJ, Cock IE. A review of the traditional uses, medicinal properties and phytochemistry of *Centaurea benedicta* L. Pharmacogn J 2021; 13: 798–812

[2] Ulbricht C, Basch E, Dacey C, Dith S, Hammerness P, Hashmi S, Seamon E, Vora M, Weissner W. An evidence-based systematic review of blessed thistle (*Cnicus benedictus*) by the natural standard research collaboration. J Diet Suppl 2008; 5: 422

[3] Al-Snafi E. The constituents and pharmacology of *Cnicus Benedictus*–A review. Pharm Chem J 2016; 3: 129–135

[4] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46(1–3): 3–26

[5] Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment based contributions and its application to the prediction of drug transport properties. J Med Chem 2000; 43: 3714–3717

[6] Rai M, Singh AV, Paudel N, Kanase A, Falletta E, Kerkar P, Heyda J, Barghash RF, Pratap Singh S, Soos M. Herbal concoction unveiled: A compu-

tational analysis of phytochemicals' pharmacokinetic and toxicological profiles using novel approach methodologies (NAMs). Curr Res Toxicol 2023; 5: 100118

[7] Bero S, Muda A, Choo Y, Muda N, Pratama S. Similarity measure for molecular structure: A brief review. J Phys Conf Ser 2017; 892(1): 012015

[8] Yu R, Liu Z, Yu R, Zhang H, Shao Y, Mei L, Tao Y. A simple method for isolation and structural identification of arctigenin from Saussurea medusa Maxim. by preparation chromatography and single crystal X-ray diffraction. J Med Plant Res 2021; 5: 979–983. DOI: 10.5517/ccdc.csd.cc28tq4z

[9] Cho MK, Jang YP, Kim YC, Kim SG. Arctigenin, a phenylpropanoid dibenzylbutyrolactone lignan, inhibits MAP kinases and AP-1 activation via potent MKK inhibition: the role in TNF-alpha inhibition. Int Immunopharmacol 2004; 4: 1419–1429

[10] Koech PK, Jócsák G, Boldizsár I, Moldován K, Borbély S, Világi I, Dobolyi A, Varró P. Anti-glutamatergic effects of three lignan compounds: Arctigenin, matairesinol and trachelogenin – An *ex vivo* study on rat brain slices. Planta Med 2023; 89: 879–889

[11] Xiao Y, Shao K, Zhou J, Wang L, Ma X, Wu D, Yang Y, Chen J, Feng J, Qiu S, Lv Z, Zhang L, Zhang P, Chen W. Structure-based engineering of substrate specificity for pinoresinol-lariciresinol reductases. Nat Commun 2021; 12: 2828

[12] TargetMol, a drug screening expert. Accessed on March 10, 2024 at: https://www.targetmol.com/compound/trachelogenin?utm_source=-Bing&utm_medium+=CPC&utm_term=Trachelogenin&msclkid=5292e987eb6b1afceac88c3c9dcb28c6

[13] Moujir L, Callies O, Sousa PMC, Sharopov F, Seca AML. Applications of sesquiterpene lactones: A review of some potential success cases. Applied Sciences 2020; 10: 3001

[14] Bosco A, Golsteyn RM. Emerging anti-mitotic activities and other bioactivities of sesquiterpene compounds upon human cells. Molecules 2017; 22: 459

[15] Ulubelen A, Berkan T. Triterpenic and steroidal compounds of *Cnicus benedictus*. Planta Med 1977; 31: 375–377

[16] Rezig K, Benkaci-Ali F, Foucaunier ML, Laurent S, Umar HI, Alex OD, Tata S. HPLC/ESI-MS characterization of phenolic compounds from *Cnicus benedictus* L. roots: A study of antioxidant, antibacterial, anti-inflammatory, and anti-alzheimer's activity. Chem Biodivers 2024; 21: e202300724. DOI: 10.1002/cbdv.202300724

[17] Avula B, Katragunta K, Wang YH, Ali Z, Khan IA. Simultaneous determination and characterization of flavonoids, sesquiterpene lactone, and other phenolics from *Centaurea benedicta* and dietary supplements using UHPLC-PDA-MS and LC-DAD-QToF. J Pharm Biomed Anal 2022; 216: 114806

[18] Bradley PR (editor). British Herbal Compendium. Vol 1. Bournemouth: British Herbal Medicine Association; 1992: 126–127

[19] Schneider G, Lachner I. Beitrag zur Analytik und Wirkung von Cnicin [Analysis and action of cnicin]. Planta Med 1987; 53: 247–251

[20] Vanhaelen-Fastre R. Présence du Saloniténolide dans *Cnicus benedictus*. Planta Med 1974; 26: 375–379

[21] Rosselli S, Maggio A, Raccuglia RA, Bruno M. Rearrangement of germacranolides. Synthesis and absolute configuration of elemane and heliangolane derivatives from cnicin. European J Org Chem 2003; 14: 2690–2694

[22] Saleem A, Walshe-Roussel B, Harris C, Asim M, Tamayo C, Sit S, Arnason JT. Characterisation of phenolics in Flor-Essence – a compound herbal product and its contributing herbs. Phytochem Anal 2009; 20: 395–401

[23] Ivancheva S, Stantcheva B. Ethnobotanical inventory of medicinal plants in Bulgaria. J Ethnopharmacol 2000; 69: 165–172

[24] El-Hilaly J, Hmammouchi M, Lyoussi B. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). J Ethnopharmacol 2003; 86: 149–158

[25] Leporatti ML, Ivancheva S. Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. J Ethnopharmacol 2003; 87: 123–142

[26] European Medicines Agency (EMA) Accessed on 10 August 2024 at: https://www.ema.europa.eu/en/medicines/herbal/cnici-benedicti-herba

[27] Tamayo C, Richardson MA, Diamond S, Skoda I. The chemistry and biological activity of herbs used in Flor-Essence herbal tonic and Essiac. Phytother Res 2000; 14: 1–14

[28] Nice FJ. Common herbs and foods used as galactogogues. ICAN 011; 3: 129–132

[29] McBride GM, Stevenson R, Zizzo G, Rumbold AR, Amir LH, Keir AK, Grzeskowiak LE. Use and experiences of galactagogues while breastfeeding among Australian women. PLoS One 2021; 16: e0254049

[30] Bela Z. Blessed thistle – Panacea of the 16th century Europe. Panacea 2005; 1: 38–42

[31] Chabane D, Assani A, Mouhoub F, Bourakba C, Nazeli N. Anatomical, phytochemical and pharmacological studies of roots of *Cnicus benedictus* L. Int J Med Plant Res 2013; 2: 204–208

[32] Fabbri CN. Treating medieval plague: The wonderful virtues of theriac. Early Sci Med 2007; 12: 247–283

[33] Zhang J, Shen S, Zhu S, Jia F, Li J, Sun Y. *Cnicus benedictus* extract-loaded electrospun gelatin wound dressing for treating diabetic wounds: An in vitro and in vivo study. J Appl Biomater Funct Mater 2024; 22: 22808000241245298. DOI: 10.1177/22808000241245298

[34] Steenkamp V, Gouws M. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. S Afr J Bot 2006; 72: 630–633

[35] Vanhaelen-Fastre R. Constitution et propietes anti- bacteriennes de l'huile essentielle de *Cnicus benedictus*. Planta Med 1973; 24: 165–175

[36] Bach SM, Fortuna MA, Attarian R, de Trimarco JT, Catalán CA, Av-Gay Y, Bach H. Antibacterial and cytotoxic activities of the sesquiterpene lactones cnicin and onopordopicrin. Nat Prod Commun 2011; 6: 163–166

[37] Vanhaelen-Fastre R. Constituents polyacetyleniques de *Cnicus benedictus* L. Planta Med 1974; 25: 47–59

[38] Vanhaelen-Fastre R, Vanhaelen M. Activite antibiotique et cytotoxique de la cnicine et de sesproduits d'hydrolyse. Relation structure chimique – Activite biologique. Planta Med 1976; 29: 179–189

[39] Szabó I, Pallag A, Blidar CF. The antimicrobial activity of the *Cnicus benedictus* L. extracts. Analele Universitatii din Oradea, Fascicula Biologie 2009, Tom. XVI/1, 126–128. https://www.semanticscholar.org/paper/The-antimicrobial-activity-of-the-Cnicus-benedictus-Szab%C3%B3-Pallag/5723d742035dda2b6a361f941fd24838ed5de90b#citing-papers

[40] Yasin Y, Ibrahem EJ. Antibacterial activity of ethanolic extract of roots of the goo (*Cnicus benedictus* L.). Cihan Univ Sci J 2017; 2017: 298–310

[41] Raina D, Kumar C, Kumar V, Khan IA, Saran S. Potential inhibitors targeting *Escherichia coli* UDP-N-acetylglucosamine enolpyruvyl transferase (MurA): An overview. Indian J Microbiol 2022; 62: 11–22

[42] Bachelier A, Mayer R, Klein CD. Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA. Bioorg Med Chem Lett 2006; 16: 5605–5609

[43] Monsalve LN, Rosselli S, Bruno M, Baldessari A. Lipase-catalysed preparation of acyl derivatives of the germacranolide cnicin. J Mol Catal B Enzym 2009; 57: 40–47

[44] Skarzynski T, Mistry A, Wonacott A, Hutchinson SE, Kelly VA, Duncan K. Structure of UDP-N-acetylglucosamine enolpyruvyl transferase, an enzyme essential for the synthesis of bacterial peptidoglycan, complexed with substrate UDP-N-acetylglucosamine and the drug fosfomycin. Structure 1996; 4: 1465–1474

[45] Steinbach A, Skarzynski T, Scheidig AJ, Klein CD. The Antibacterial Target Enzyme Mura Synthesizes its Own Non-Covalent Suicide Inhibitor from the Natural Product Cnicin. Available online: https://wwwrcsborg/structure/2Z2C. Accessed on 10 August 2024

[46] Shigetomi K, Shoji K, Mitsuhashi S, Ubukata M. The antibacterial properties of 6-tuliposide B. Synthesis of 6-tuliposide B analogues and structure-activity relationship. Phytochemistry 2010; 71: 312–324

[47] Barrero AF, Oltra JE, Alvarez M, Raslan DS, Saúde DA, Akssira M. New sources and antifungal activity of sesquiterpene lactones. Fitoterapia 2000; 71: 60–64

[48] Barrero AF, Oltra JE, Raslan DS, Saude DA. Microbial transformation of sesquiterpene lactones by the fungi cunninghamella echinulata and rhizopus oryzae. J Nat Prod 1999; 62: 726–729

[49] Minoda K, Nagaoka Y, Uesato S, Hayashi K, Hayashi T. Synergistic effect of oseltamivir and arctigenin from seeds (Goboshi) of *Arctium lappal* on Anti-influenza A Virus Activity. Symposium on the chemistry of natural products. 2006; P-206

[50] Hayashi K, Narutaki K, Nagaoka Y, Hayashi T, Uesato S. Therapeutic effect of arctiin and arctigenin in immunocompetent and immunocompromised mice infected with influenza A virus. Biol Pharm Bull 2010; 33: 1199–1205

[51] So J, Kim JH, Lee S, Kim C, Park R, Park J. Arctigenin from *Forsythia viridissima* fruit inhibits the replication of human coronavirus. Int J Mol Sci 2024; 25: 7363. DOI: 10.3390/ijms25137363

[52] Alhadrami HA, Sayed AM, Hassan HM, Youssif KA, Gaber Y, Moatasim Y, Kutkat O, Mostafa A, Ali MA, Rateb ME, Abdelmohsen UR, Gamaleldin NM. Cnicin as an anti-SARS-CoV-2: An integrated in silico and in vitro approach for the rapid identification of potential COVID-19 therapeutics. Antibiotics (Basel) 2021; 10: 542

[53] Queiroz LS, Ferreira EA, Mengarda AC, Almeida ADC, Pinto PF, Coimbra ES, de Moraes J, Denadai ÂML, Da Silva Filho AA. In vitro and in vivo evaluation of cnicin from blessed thistle (*Centaurea benedicta*) and its inclusion complexes with cyclodextrins against *Schistosoma mansoni*. Parasitol Res 2021; 120: 1321–1333

[54] Ahmadimoghaddam D, Sadeghian R, Ranjbar A, Izadidastenaei Z, Mohammadi S. Antinociceptive activity of *Cnicus benedictus* L. leaf extract: A mechanistic evaluation. Res Pharm Sci 2020; 15: 463–472

[55] De Sousa OV, Gonçalves GC, Queiroz LS, Ferreira EA, Santos BCS, Silva Filho AA, Taranto AG. Cnicin from *Centaurea benedicta* L. is an active compound against skin inflammation in a mouse model. 2021. DOI: 10.21203/rs.3.rs-1124507/v1

[56] Demiroz T, Albayrak G, Nalbantsoy A, Gocmen B, Baykan S. Anti-inflammatory properties of *Centaurea calolepis Boiss* and cnicin against *Macrovipera lebetina obtusa* (Dwigubsky, 1832) and *Montivipera xanthina* (Gray, 1849) venoms in rat. Toxicon 2018; 152: 37–42

[57] Gobrecht P, Gebel J, Leibinger M, Zeitler C, Chen Z, Gründemann D, Fischer D. Cnicin promotes functional nerve regeneration. Phytomedicine 2024; 129: 155641. doi:DOI: 10.1016/j.phymed.2024.155641.

[58] Li H, Zuo J, Tang W. Phosphodiesterase-4 inhibitors for the treatment of inflammatory diseases. Front Pharmacol 2018; 9: 1048

[59] Li H, Li J, Zhang X, Feng C, Fan C, Yang X, Zhang R, Zhu F, Zhou Y, Xu Y, Liu H, Tang W. DC591017, a phosphodiesterase-4 (PDE4) inhibitor with robust anti-inflammation through regulating PKA-CREB signaling. Biochem Pharmacol 2020; 177: 113958

[60] Li H, Zhang X, Xiang C, Feng C, Fan C, Liu M, Lu H, Su H, Zhou Y, Qi Q, Xu Y, Tang W. Identification of phosphodiesterase-4 as the therapeutic target of arctigenin in alleviating psoriatic skin inflammation. J Adv Res 2021; 33: 241–251

[61] Nose M, Fujimoto T, Nishibe S, Ogihara Y. Structural transformation of lignan compounds in rat gastrointestinal tract; II. Serum concentration of lignans and their metabolites. Planta Med 1993; 59: 131–134

[62] Mascolo N, Autore G, Capasso F, Menghini A, Fasulo MP. Biological screening of Italian medicinal plants for antiinflammatory activity. Phytother Res 1987; 1: 28–31

[63] Durdun C, Papuc C, Crivineanu M, Nicorescu V. Antioxidant potential of *Lycopodium clavatum* and *Cnicus benedictus* hydroethanolic extracts on stressed mice. Sci Works. C Series. Vet Med 2011; 57: 61–68

[64] Jang YP, Kim SR, Choi YH, Kim J, Kim SG, Markelonis GJ, Oh TH, Kim YC. Arctigenin protects cultured cortical neurons from glutamate-induced neurodegeneration by binding to kainate receptor. J Neurosci Res 2002; 68: 233–240. doi:DOI: 10.1002/jnr.10204

[65] Kang HS, Lee JY, Kim CJ. Anti-inflammatory activity of arctigenin from Forsythiae Fructus. J Ethnopharmacol 2008; 116: 305–312. doi:DOI: 10.1016/j.jep.2007.11.030

[66] Zhao F, Wang L, Liu K. In vitro anti-inflammatory effects of arctigenin, a lignan from *Arctium lappa* L., through inhibition on iNOS pathway. J Ethnopharmacol 2009; 122: 457–462

[67] Zhang W, Jiang Z, He B, Liu X. Arctigenin protects against lipopolysaccharide-induced pulmonary oxidative stress and inflammation in a mouse model via suppression of MAPK, HO-1, and inos. signaling, Inflammation 2015; 38: 1406–1414

[68] Erel SB, Karaalp C, Bedir E, Kaehlig H, Glasl S, Khan S, Krenn L. Secondary metabolites of *Centaurea calolepis* and evaluation of cnicin for anti-inflammatory, antioxidant, and cytotoxic activities. Pharm Biol 2011; 49: 840–849

[69] Qi Y, Dou DQ, Jiang H, Zhang BB, Qin WY, Kang K, Zhang N, Jia D. Arctigenin attenuates learning and memory deficits through PI3 k/Akt/GSK-3β pathway reducing tau hyperphosphorylation in Aβ-induced AD mice. Planta Med 2017; 83: 51–56

[70] Shri SR, Manandhar S, Nayak Y, Pai KSR. Role of GSK-3β inhibitors: New promises and opportunities for alzheimer's disease. Adv Pharm Bull 2023; 13: 688–700

[71] Kim JY, Hwang JH, Cha MR, Yoon MY, Son ES, Tomida A, Ko B, Song SW, Shin-ya K, Hwang Y. Arctigenin blocks the unfolded protein response and shows therapeutic antitumor activity. J Cell Physiol 2010; 224: 33–40

[72] Awale S, Lu J, Kalauni SK, Kurashima Y, Tezuka Y, Kadota S, Esumi H. Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Res 2006; 66: 1751–1757

[73] Takasaki M, Konoshima T, Komatsu K, Tokuda H, Nishino H. Anti-tumor-promoting activity of lignans from the aerial part of *Saussurea medusa*. Cancer Lett 2000; 158: 53–59

[74] Hirose M, Yamaguchi T, Lin C, Kimoto N, Futakuchi M, Kono T, Nishibe S, Shirai T. Effects of arctiin on PhIP-induced mammary, colon and pancreatic carcinogenesis in female Sprague-Dawley rats and MeIQx-induced hepatocarcinogenesis in male F344 rats. Cancer Lett 2000; 155: 79–88

[75] Sen A, Ozbas Turan S, Bitis L. Bioactivity-guided isolation of anti-proliferative compounds from endemic *Centaurea kilaea*. Pharm Biol 2017; 55: 541–546

[76] Jöhrer K, Obkircher M, Neureiter D, Parteli J, Zelle-Rieser C, Maizner E, Kern J, Hermann M, Hamacher F, Merkel O. Antimyeloma activity of the sesquiterpene lactone cnicin: Impact on Pim-2 kinase as a novel therapeutic target. J Mol Med (Berl) 2012; 90: 681–693. doi:DOI: 10.1007/s00109-011–0848-x

[77] Barrero AF, Oltra JE, Morales V, Alvarez M, Rodríguez-García I. Biomimetic cyclization of cnicin to malacitanolide, a cytotoxic eudesmanolide from *Centaurea malacitana*. J Nat Prod 1997; 60: 1034–1035

[78] Matsuzaki Y, Koyama M, Hitomi T, Yokota T, Kawanaka M, Nishikawa A, Germain D, Sakai T. Arctiin induces cell growth inhibition through the down-regulation of cyclin D1 expression. Oncol Rep 2008; 19: 721–727

[79] Hirano T, Gotoh M, Oka K. Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. Life Sci 1994; 55: 1061–1069

[80] Zeller W, de Gols M, Hausen BM. The sensitizing capacity of *Compositae* plants. VI. Guinea pig sensitization experiments with ornamental plants and weeds using different methods. Arch Dermatol Res 1985; 277: 28–35

[81] Blessed Thistle. 2022 Feb 20. In: Drugs and Lactation Database (LactMed®) [Internet]. Bethesda (MD): National Institute of Child Health and Human Development; 2006 – PMID: 30000834

[82] OECD Guidelines for the Testing of Chemicals, Section 4. Accessed on 10 August 2024 at: https://wwwoecd-ilibraryorg/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

[83] Maharjan RS, Singh AV, Hanif J, Rosenkranz D, Haidar R, Shelar A, Singh SP, Dey A, Patil R, Zamboni P, Laux P, Luch A. Investigation of the associations between a nanomaterial's microrheology and toxicology. ACS Omega 2022; 7: 13985–13997

[84] Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017; 7: 42717

[85] Cheng T, Zhao Y, Li X, Lin F, Xu Y, Zhang X, Li Y, Wang R, Lai L Computation of octanol water partition coefficients by guiding an additive model with knowledge. J Chem Inf Model 2007; 47: 2140–2148

[86] Wildman SA, Crippen GM. Prediction of physicochemical parameters by atomic contributions. J Chem Inf Model 1999; 39: 868–873

[87] Moriguchi I, Shuichi H, Liu Q, Nakagome I, Matsushita Y. Simple method of calculating octanol/water partition coefficient. Chem Pharm Bull 1992; 40: 127–130

[88] Moriguchi I, Shuichi H, Nakagome I, Hirano H. Comparison of reliability of log P values for drugs calculated by several methods. Chem Pharm Bull 1994; 42: 976–978

[89] Daina A, Zoete V. A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem 2016; 11: 1117–1121

[90] Suenderhauf C, Hammann F, Huwyler J. Computational prediction of blood-brain barrier permeability using decision tree induction. Molecules 2012; 17: 10429–10445

[91] Singh AV, Chandrasekar V, Laux P, Luch A, Dakua SP, Zamboni P, Shelar A, Yang Y, Pandit V, Tisato V, Gemmati D. Micropatterned neurovascular interface to mimic the blood-brain barrier's neurophysiology and micromechanical function: A BBB-on-CHIP model. Cells 2022; 11: 2801

[92] Nabeka H. Prosaposin, a neurotrophic factor, protects neurons against kainic acid-induced neurotoxicity. Anat Sci Int 2021; 96: 359–369

[93] Daina A, Michielin O, Zoete V. SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res 2019; 47: W357–W364

[94] Gfeller D, Michielin O, Zoete V. Shaping the interaction landscape of bioactive molecules. Bioinformatics 2013; 29: 3073–3079

[95] Wu D, Jin L, Huang X, Deng H, Shen QK, Quan ZS, Zhang C, Guo HY. Arctigenin: Pharmacology, total synthesis, and progress in structure modification. J Enzyme Inhib Med Chem 2022; 37: 2452–2477

[96] Mizuno S, Ikegami M, Koyama T, Sunami K, Ogata D, Kage H, Yanagaki M, Ikeuchi H, Ueno T, Tanikawa M, Oda K, Osuga Y, Mano H, Kohsaka S. High-throughput functional evaluation of MAP2K1 variants in cancer. Mol Cancer Ther 2023; 22: 227–239

[97] Xie LH, Ahn EM, Akao T, Abdel-Hafez AA, Nakamura N, Hattori M. Transformation of arctiin to estrogenic and antiestrogenic substances by human intestinal bacteria. Chem Pharm Bull (Tokyo) 2003; 51: 378–384