CHAPTER - II

REVIEW OF LITERATURE

This chapter consolidated previous studies and make outline for the foundation of future research by critically analysed the literature on *Cynanchum* species, yet there is no previous report was published on *Cynanchum tunicatum*. It comprises plant tissue culture techniques, phytochemical profiling, compound isolation, biological activities and molecular docking related to cancer studies.

2.1. Plant description

Cynanchum tunicatum Retz. (Alston) is a rare medicinal plant (Praveen kumar, 2018) and it is a climber that belongs to the family Apocynaceae. It is commonly called as dog-strangling wine or milk weed. In Tamil, it is called as Aattu Moola Kodi. There are roughly 2980 species in 315 genera in this family. The genus *Cynanchum* has approximately 200 species, which have been used in folk medicine such as an antifebrile, antitumor, diuretic, anodyne, tonic, and chronic hepatitis (Tawfiq et al., 1991). Steroids, alkaloids, terpenoids, flavonoids, phenols, polysaccharides, and glycosides were approximately 300 compounds identified from the genus *Cynanchum* (Shan et al., 2006).

2.2. Systematic position

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledonae

Sub class: Gamopetalae

Series: Bicarpellate

Order: Genetianales

Family: Apocynaceae

Genus: *Cynanchum*

Species: *tunicatum*

Binominal Name: *Cynanchum tunicatum* (Retz.) Alston

2.3. Medicinal uses from other species

Wang et al. observed *Lycium barbarum* using *Cynanchum komarovii* extracts and eucalyptus oil-loaded microcapsules (EOMCs) formulation, which is eco-friendly, long-term, and low-risk aphid control. The optimization of the composition is effective for control of aphids, the release of EOMCs was controlled by changing the cross-linking degree of the shell to match the aphid control characteristics of *C. komarovii* extracts. Four types of polyamines were used as cross-linking agents for the preparation of EOMCs by interfacial polymerization. The bioactivity, wettability, and field application efficacy of *C. komarovii* extracts and different EOMCs formulations were evaluated. These EOMCs exhibited an encapsulation efficiency exceeding 85%. The control efficiency of the compositions of microcapsules with a moderate release rate and *C. komarovii* extracts on aphids remained at 62.86%, while the control efficiency of of microcapsules with the fastest and slowest rates with *C. komarovii* extracts was only 48.62% and 57.11%, respectively. The formulation of *C. komarovii* extracts with all four types of EOMCs were found to be safe for *L. barbarum*. The selection of appropriate polyamines during fabrication, the release rate can be effectively controlled to achieve sustainable and low-risk aphid control in *L. barbarum* through selected microcapsules (Wang et al., 2024).

In *Cynanchum auriculatum,* twenty chromatographic peaks were identified such as Steroids (C_{21}), organic acids, coumarin, acetophenone and compared with reference compounds. Totally, 39 migratory compounds were discovered in CA-containing plasma, which was found to significantly promote the contractility of the isolated duodenum. Moreover, multivariate analysis of the spectrum-effect in CA-containing plasma were significantly associated with the anti-FD effect. These compounds included seven prototype compounds such as cynanoneside A, syringic acid, deacylmetaplexigenin, ferulic acid, scopoletin, baishouwubenzophenone, and qingyangshengenin. The inhibition of ABC transporters demonstrated that the inhibitors verapamil and Ko143 significantly increased ($P < 0.05$) the uptake of scopoletin and qingyangshengenin (Sun et al., 2024).

Patients with sepsis are at an incremental risk of Acute Lung Injury (ALI). Baiqian, also known as *Cynanchum stauntonii*, has an anti-inflammatory property and is traditionally used to treat cough and phlegm. The demonstration of multicomponent, multitarget, and multi-pathway regulatory molecular mechanisms of Csrer in treating Lipopolysaccharide (LPS) induced ALI. The bioactive components of Csrer were identified by ultra-high performance liquid chromatography Q-Orbitrap mass spectrometry (UPLC-Q-Orbitrap MS). Active targets predicted from Pharm Mapper. Drug Bank, OMIM, TTD, and Gene Cards were used to identify potential targets related to ALI. Intersection genes were identified for Csrer against ALI. The PPI network was analysed to identify prime targets. GO and KEGG analyses were performed. A drug-compound-target pathway-disease network was constructed. Molecular docking and simulations evaluated the binding free energy between key proteins and active compounds. The protective effect and mechanism of Csrer in ALI were verified using an ALI model in mice. WB, RT-PCR, and immunofluorescence evaluated the mechanisms of the pulmonary protective effects of Csrer. Forty-six bioactive components, 100 and 92 potential cross-targets against ALI and 10 core genes were identified. According to GO and KEGG analyses, the PI3K-Akt, apoptosis and p53 pathways are predominantly involved in the "Csrer-ALI" network. According to molecular docking and dynamics simulations, 10 key genes were firmly bound by the principal active components of Csrer. The "Csrer-ALI" network was revealed the p53-mediated apoptosis and inflammatory pathways in animal experiments. Csrer is a reliable source for ALI treatment based on its practical components, potential targets and pathways (Hejun et al., 2024).

According to Huang et al. Cynatratoside-C (CyC), a C-21 steroidal saponin isolated from *Cynanchum atratum*, is a potential candidate for treating freshwater fish infected with

ciliated parasite *Ichthyophthirius multifiliis*. To promote the drug development process of CyC, the stability of CyC were analysed with the help of heating, sterile water, and aquaculture water treatments. An accelerated stability test demonstrated the thermal degradation kinetics followed by first-order reaction with a predicted half-life of 3472 days (9.5 years) at a temperature of 25°C. According to the Arrhenius model, the activation energy of CyC was calculated as 91.7 kJ/mol. When CyC was stored in sterile water for 3 years, the mortality rates of theronts and nonencysted tomonts were decreased significantly in the low CyC concentration treatment group, and the minimum concentration that inhibited the production of encysted tomonts increased from 0.06 mg/L to 0.125 mg/L. When CyC was stored in sterile water for 6 years, it showed a nearly complete loss of antiparasitic efficacy, indicated that water may promote the degradation of CyC. When CyC was stored in aquaculture water, the antiparasitic efficacy of CyC against theronts and tomonts was decreased significantly at 2 days post-storage, specified that microorganisms in aquaculture water could absorb and degrade CyC (Huang et al., 2024).

The polysaccharides of *Cynanchum auriculatum* (CAP), which were degraded by single-enzymatic method (α-amylase) and double-enzymatic method (α-amylase and glucoamylase) were compared. CAP had good water solubility and higher non-starch polysaccharide content. A homogeneous neutral polysaccharide CAP-W, with the degree of acetylation about 17 %, was obtained from CAP by anion exchange column chromatography. CAP-W, with the weight average molecular weight of 8.4 kDa, was composed of mannose, glucose, galactose, xylose, and arabinose in a molar ratio of 1.27:1.00:0.25:0.10:1.16. The backbone included *β*-1,4-Man*p*, *β*-1,4,6-Man*p*, *β*-1,4-Glc*p* and *β*-1,4,6-Glc*p* residues, with branches at the O-6 position of *β*-1,4,6-Man*p* and *β*-1,4,6-Glc*p* residues, consisted of *α*-T-Ara*f*, *α*-1,5-Ara*f*, *α*-1,2,5-Ara*f*, *α*-1,3,5-Ara*f*, T-Xyl*p*,1,4-Xyl*p*, *β*-T-Man*p* and *β*-T-Gal*p* residues. *In vitro* immunological experiments suggested that CAP-W improved the phagocytic ability of macrophages, stimulated the release of NO, TNF-α and IL-6 from RAW264.7 cells, promoted the expression of NF-κB and caused nuclear translocation of NF-κB p65 (Wang et al., 2023).

The antifungal activity of *Cynanchum komarovii* extracts prepared using ethanol, petroleum ether, ethyl acetate and n‐butyl alcohol. It showed that ethanol extract (50 mg/mL) had inhibitory effect against *Fusarium semitectum*, *Rhizoctonia solani*, *Setosphaeria turcica, Botrytis cinerea* and *Valsa mali,* the inhibition percentage ranged from 44.0 to 61.2%. The antifungal activity of petroleum ether, ethyl acetate and n‐butyl alcohol exhibited the

inhibition percentage ranged from 76.4 to 100.0%. A compound was isolated from n‐butyl alcohol extract by column chromatography and characterized as 7‐demethoxylophorine by compared spectral data and physical properties. 7‐Demethoxylophorine had significant inhibition against *F. semitectum*, with EC_{50} values of 4.6637 μg/mL, and that could be used as a new bio-fungicide against *F. semitectum* (Wang et al., 2023).

Cynanotophyllosides E-F, two new minor pregnane glycosides were isolated from the antidepressant active fraction of *Cynanchum otophyllum*, and their IUPAC names were determined as 12-*O*-vanilloyl-deacetylmetaplexigenin 3-*O*-ββ-D-glucopyranosyl-(1→4)-ββ-D-glucopyranosyl-(1→4)-ββ-D-cymaropyranosyl-(1→4)-ββ-D-oleandropyranosyl-(1→4)-ββ-D-digitoxopyranoside, and 12-*O*-nicotinoyl-deacetylmetaplexigenin 3-*O*-ββ-D-glucopyranosyl-(1→4)-ββ-D-glucopyranosyl-(1→4)-ββ-D-cymaropyranosyl-(1→4)-ββ-D-oleandropyranosyl-(1→4)-ββ-D-cymaropyranoside respectively, with spectroscopic analysis (Li et al., 2023).

Steroidal glycosides (C21) are a group of natural compounds with biological activities such as anti-cancer, anti-microbial, and anti-viral properties. The isolation and determination of the structure of Marsdenialongise A, a new C21 steroidal glycoside from *Marsdenia longipes*, using nuclear magnetic resonance spectroscopy and mass spectra data. Marsdenialongise A is a derivative of tenacigenin B and was isolated for the first time from a plant. The inhibitory effect of Marsdenialongise A on cancer cells was evaluated through MTT and cell migration assays, cell cycle, and apoptosis analyses. It showed that Marsdenialongise A reduces the cell viability of cancer cells, with the AGS cell line are more sensitive than other cell lines, with an IC_{50} value of 5.69 μM (for 48 h of treatment). Marsdenialongise A also exhibited an ability to prevent the migration of cancer cells in AGS cells. Further analysis using flow cytometry has revealed that Marsdenialongise A is capable of inducing cell cycle arrest and apoptosis. The overexpression of reactive oxygen species (ROS) production induced by Marsdenialongise A can be leads to the influence on cell cycle and apoptosis of cancer cells. Thus, Marsdenialongise A can be considered a potential anti-cancer agent (Le et al., 2023).

Cynanchum auriculatum is native to Asia, possessed significant nutritional and health benefits. However, the presence of Cadmium (Cd) in the soil poses a hazard to the germination and growth of *C. auriculatum*. As Nitric oxide (NO) plays a vital role in plant resistance to heavy metal stress, three different concentrations of silver nanoparticle (SNP) treatment during the germination phase, aimed to alleviate the inhibitory effects of Cd stress on the seed

germination of *C. auriculatum*. It indicated that when compared to seeds treated with SNP concentrations of 0.2 mM and 0.8 mM, *C. auriculatum* seeds treated with 0.4 mM SNP exhibited an improved germination rate and germination index, as well as longer hypocotyl. Furthermore, in comparison to NOS, the SNP application stimulated the production of NO through NR catalysis. Additionally, ABA level decreased while GA level increased under normal conditions, while SNP application enhanced the accumulation of both ABA and GA in *C. auriculatum* seeds under Cd stress. Histochemical staining and biochemical indicators demonstrated that SNP treatment enhanced the enzymatic activity of SOD, POD, and CAT, while inhibited the production of hydrogen peroxide and superoxide anion. Moreover, SNP treatment resulted in increased α-amylase activity, which facilitated starch hydrolysis and soluble sugar. Ultimately, the seed vitality of *C. auriculatum* under Cd stress was promoted (Liu et al., 2023).

Cynanchum bungei is an agricultural crop with a high starch content which contain biological activities such as anti-tumor, anti-depressant, anti-oxidant etc. Three concentrations of *C. bungei*, namely, 5%, 15%, and 25%, were added to media to *C. bungei* rice wine. The basic physical and chemical properties, antioxidant activities, sensory characteristics, and volatile components of *C. bungei* rice wine was determined. The effects of *C. bungei* extract on the cell viability, alcohol dehydrogenase activity, and glucose absorption capacity of *Saccharomyces cerevisiae* were analyzed. The main bioactive compound showed antioxidant activity of the rice wine were increased. However, the vitality of *S. cerevisiae* was inhibited by *C. bungei*. Combined with a sensory evaluation, 15% *C. bungei* was found to be the optimal additive concentration with which to brew *C. bungei* rice wine (Cai et al., 2023).

Response surface methodology was used to optimize the extraction process of steroidal glycosides from *Cynanchum auriculatum* by complex enzymatic method, and its effects on alcohol dehydrogenase activity and human hepatoma cell HepG2 were analyzed. The alcohol-induced hepatocyte damage model of HepG2 was used to investigate the contents of ALT, LDH, and GSH and changes in cell shape. The optimum extraction conditions of steroidal glycoside enzyme were obtained at an enzymolysis duration of 100 min, a temperature of 46°C, enzyme dose of 1.30% and pH of 5.0. Consequently, the actual extraction rate of 2.80±0.04% was achieved using the optimized process conditions, which was close to the expected value. Compared with the model group, it significantly suppresses the growth of ALT and LDH while increasing the amount of intracellular GSH, and the cell damage was restored. The compound

enzyme method can effectively extract steroidal glycosides of *C. auriculatum* have hepatoprotective effect (Wan-yu et al., 2023).

Based on traditional pharmacological applications and partial *in vitro* data, *Cynanchum atratum* (CA) is proposed to act on skin whitening. The anti-melanogenesis activity of CA fraction B (CAFB) on UVB-induced skin hyperpigmentation. Forty C57BL/6j mice were exposed to UVB (100 mJ/cm^2, five times/week) for eight weeks. After irradiation, CAFB was applied to the left ear once a day for 8 weeks (the right ear served as an internal control). The CAFB significantly reduced melanin production in the ear skin, as indicated by the gray value and Mexameter melanin index. In addition, CAFB treatment notably decreased melanin production in α-MSH-stimulated B16F10 melanocytes, along with a significant reduction in tyrosinase activity. Cellular cAMP (cyclic adenosine monophosphate), MITF (microphthalmia-associated transcription factor), and tyrosinase-related protein 1 (TRP1) were also notably downregulated by CAFB. CAFB is a potential ingredient for treating skin disorders caused by the overproduction of melanin and its underlying mechanisms involved the modulation of tyrosinase, mainly mediated by the regulation of the cAMP cascade and MITF pathway (Wang et al., 2023).

A novel actinobacterial strain, SB3-54^T was isolated from rhizosphere soil of *Cynanchum wilfodill*. Cells of strain SB3-54^T were gram-stain-positive, aerobic, rod-shaped, and flagellated which formed pale yellow colonies on Reasoners 2A agar. Growth occurred at 15–30 °C, pH 5.8, and 0–2.5% NaCl. Phylogenetic and phylogenomic analyses showed that strain SB3-54^T formed a separate lineage in the *Jatrophihabitans telluris* N237^T. Strain SB3-54^T was positive for catalase activity. Genomic analysis showed that SB3-54^T has plant beneficial functions such as root colonization and plant protection from oxidative stress. Furthermore, genome of SB3-54^T contained gene clusters related to cytokinin biosynthesis, auxin response, tryptophan biosynthesis, siderophore biosynthesis and bacterial toxin-antitoxin systems. Strain SB3-54^T contained iso-$C_{16:0}$ as the major fatty acid and MK-9(H_4) and MK-9(H_6) as the predominant quinones. The organism had *meso*-diaminopimelic acid as the diagnostic diamino acid in the peptidoglycan. The major polar lipids such as diphosphatidylglycerol, phosphatidylinositol polymannosides, two unidentified aminoglycophospholipids and three unidentified phospholipids (Suh et al., 2024).

The fallen leaf litter of *Cynanchum auriculatum* (CA) caused the continuous cropping obstacle (CCO) through growth promotion and invasion reinforcement of soil-borne fungal

pathogen. Water extract of Leaf (LE) and root extracts (RE) were compared for their effects on seed germination, seedling growth indices, and plant defense enzymes activities. The effects of LE on soil microbial communities were determined by using high-throughput sequencing technology. A fungal strain D1 belongs to *Fusarium solani* which cause root rot disease. It was isolated and confirmed for its potential contribution to CCO. Both LE and RE inhibited seed germination, seedling growth, and plant defense enzymes activities. LE coupled with fungal strain D1 aggravated the impacts of CCO. Apart from the induction of propagation of D1 in soil, extracts could also promote hypha weight, spore number, and spore germination rate of fungal strain D1 under the culture conditions. Compared with RE, LE showed more promoting-effects on the pathogenesis-related enzyme activity of D1. Moreover, possible active substances such as caffeic acid and ferulic acid were contributed. Besides, fungal community were shifted by LE+D1. It suggested that water extract of leaf litter promoted the growth and propagation of strain D1, and enhanced its pathogenicity towards *C. auriculatum*, which synthetically contributed to the CCO process (Shen et al., 2023).

The complete chloroplast genome of *Cynanchum acutum* using high-throughput Illumina sequencing reads. The chloroplast genome assembly displayed a typical quadripartite structure with a total length of 158,283 bp, which contained a pair of inverted repeats (IR) regions of 24,459 bp. These two IRs were separated by a large single-copy region and a small single-copy region of 89,424 bp and 19,941 bp in length, respectively. The *C. acutum* genome contained 130 genes, and its overall GC content was 37.87%. Phylogenetic analysis among *C. acutum* and nine other *Cynanchum* species demonstrated that *C. acutum* was closely related to *C. chinense* (Zhang et al., 2023).

The species *Cynanchum wilfordii* and *Cynanchum auriculatum* resembles in characteristic of fruit and root. So, it is difficult for public to recognize these two species. The images were collected to categorize *C. wilfordii* and *C. auriculatum*, which were then processed and input into a deep-learning classification model. The 200 photographs were obtained from each species, approximately 800 images were employed were used to construct a deep-learning classification model through image augmentation. For the classification, the structures of Inception-ResNet and VGGnet-19 among convolutional neural network models were used, with Inception-ResNet outperforming VGGnet-19 in terms of performance and learning speed. The validation set confirmed a strong classification performance of approximately 0.862. Furthermore, explanatory properties were added to the deep-learning model using local interpretable model-agnostic explanation (LIME), and the suitability of the

LIME domain was assessed using cross-validation in both situations. Thus, artificial intelligence may be used as an auxiliary metric in the sensory evaluation of medicinal materials in future, owing to its explanatory ability (Jung et al., 2023).

A sensitive, rapid, and reliable UPLC–ESI–MS/MS method was established, and applied for the quantification of four active components in *Cynanchum auriculatum* (CA) extract in plasma and tissue samples of normal and FD rats. After oral administration, the active components from CA (qingyangshengenin, baishouwu benzophenone, deacylmetaplexigenin, and syringic acid) were quickly absorbed and widely distributed in various tissues. The deacylmetaplexigenin and qingyangshengenin had higher absorption and lower elimination in the FD group, which indicated higher accumulation of the components in target organs for anti–FD effects. However, the baishouwu benzophenone had lower absorption and higher elimination in the FD group. Furthermore, the volume of distribution of qingyangshengenin, deacylmetaplexigenin, and syringic acid was high in the stomach and small intestine, indicated that these could be the main components mediated the pharmacodynamics of FD. In addition, the brain may be another target organ of CA for the treatment of FD, as the gut–brain axis can influence the pathogenesis of FD. The four components may alter gastrointestinal hormone levels by regulating the gut-brain axis to mediate an anti–FD effect (Sun et al., 2023).

During surveys of plant pathogens in the desert regions of Xinjiang Province, China, a leaf spot was observed from *Cynanchum sibiricum*. The suspected fungus was isolated and identified using morphology and molecular phylogeny. Morphologically, the fungus was similar to *Neodidymelliopsis cynanchi* species. Phylogenetic analyzes of combined internal transcribed spacer (ITS); the large subunit of the ribosomal rDNA (LSU); RNA polymerase II the second largest subunit (*rpb2*), and β-tubulin gene (*tub2*) sequences showed that identified fungus formed a distinct monophyletic lineage of *N. cynanchi*. The new clade is identified as *N. cynanchi* based on the phylogenetic and morphological evidence. In addition, *N. urticae* is synonymized with *N. cannabis* based on morphology and phylogeny (Qian et al., 2023).

Postmenopausal women experience several symptoms, including inflammation and a sharp rise in oxidative stress caused by estrogen deprivation. Although estrogen replacement therapy is generally regarded as an effective treatment for menopause, it has been used less frequently due to some adverse effects and high costs. Therefore, there is an immediate need to develop an effective herbal-based treatment that is affordable for low-income populations. The methanol extracts from *Cynanchum wilfordii* and *Poligonum multiflorum* (PM), two

important medicinal plants in Republic of Korea, Japan, and China were analysed for estrogen properties. In phytochemical analysis, gallic acid, 2,3,5,4′-tetrahydroxystilbene-2-O-glucoside (TSG) and emodin were quantified using HPTLC. Estrogen activity was assessed using E-screen test and gene expression analysis in estrogen receptor (ER)-positive MCF7 cells. ROS (reactive oxygen species) inhibition and anti-inflammatory activity were analyzed using HaCaT and Raw 264.7 cells, respectively. The PM extracts significantly increased the expression of the estrogen-dependent genes (ERα, ERβ, pS2) and boosted MCF7 cell proliferation in comparison to *C. wilfordii* extract. Additionally, PM extract demonstrated a significant reduction in ROS production as well as an enhanced antioxidant profile compared to *C. wilfordii* extract. Further, the PM extract treatment significantly reduced the generation of nitric oxide in RAW 264.7 cells, a murine macrophage cell line, demonstrated the anti-inflammatory properties of the extracts. It offers an experimental foundation for the use of PM extract as a phytoestrogen to minimize menopausal symptoms (Akter et al., 2023).

Cynanchum wilfordii and *Humulus lupulus* (CWHL) have been used for their various pharmacological properties in South Korea as a traditional medicine, health functional food, and their intake may relieve menopausal symptoms. The effect of compound of CWHL in menopausal symptoms of ovariectomized mice. Ovariectomized mice received CWHL or caudatin (an active ingredient of CWHL) once daily for 7 weeks. Values for hypothalamic serotonin (5-HT), dopamine, norepinephrine, estrogen receptor (ER)-β, 5-HT1A, and 5-HT2A were significantly enhanced, while value for hypothalamic monoamine oxidase A was reduced in CWHL and caudatin groups compared with ovariectomized group. CWHL and caudatin significantly reduced tail skin temperature and rectal temperature of ovariectomized mice through partial recovering of the levels of serum estrogen, nitric oxide, follicle-stimulating hormone, luteinizing hormone, and receptor-activator of the NF-κB ligand (RANKL). Moreover, CWHL and caudatin improved bone mineral density through decreasing levels of serum RANKL, tartrate-resistant acid phosphatase, and collagen type 1 cross-linked N-telopeptide and improving levels of serum alkaline phosphatase, osteoprotegerin, and osteocalcin compared with the ovariectomized group without adverse effects such as dyslipidemia. CWHL increased uterine ER-β levels but did not change uterus and vaginal weights. CWHL relieved menopausal symptoms by controlling depression, hot flashes, and osteoporosis-associated biomarkers. Therefore, CWHL might be a safe and potential candidate for management of menopause as a health functional food (Kang et al., 2023).

The effect of nitrogenous heterocyclic ester group on antitumor activities of naturally occurred C_{21}-steroidal aglycone, a series of novel 3β-nitrogenous heterocyclic ester derivatives of caudatin, kidjoranin, qingyangshengenin, and rostratamin were synthesized. The synthetic derivatives showed moderate to significant cytotoxic activities against four human cancer cells (MCF-7, HCT-116, HeLa, and HepG2), in which compounds 1s and 2c with piperidine-4-acetyl substituent exhibited the highest cytotoxic activities against the four human cancer cells with IC_{50} values ranging from 3.30 to 6.73 μM and from 3.07 to 6.37 μM, respectively. Further, *in vitro* antitumor mechanism explored that compound 2c was effective in inducing apoptosis and arrest the cell cycle in S phase in a dose- dependent manner (Li et al., 2023).

A two-step gram-scale synthesis of cynandione A. The key to success is the one-pot tandem oxidation/regioselective arylation of 1,4-hydroquinone in the presence of an excess amount of oxidant. Natural bond orbital charge analysis was performed in order to understand the regioselectivity of the arylation step. The highly practical and scalable synthesis developed herein is expected to assist the in-depth biological evaluation of cynandione A in various animal models (YoungáKo & HyunáKim, 2023).

The *in vitro* experiments were carried out in *Cynanchum komarovii* showed that 10% CK-containing serum decreased the expression level of Bcl-2, increased the expression levels of bax and cleaved Caspase-3 in synovial cells, and prevented TNF-α induced aberrant proliferation and apoptotic antagonism in HFLS-RA cells. According to *in vivo* studies, CK extract at doses above 250 mg/kg was effective in controlling the levels of inflammatory factors, lowered the arthritis index, and improved foot swelling in CIA rats. When administered at doses up to 1000 mg/kg, CK extract significantly improved synovial lesions, increased bone density, and decreased abnormally elevated immune organ index in CIA rats (Hao et al., 2023).

A phytochemical investigation on aerial parts of *Cynanchum auriculatum*, sixteen compounds were isolated which include alkaloids, coumarins, lignans, phenolic acids and flavonoids. The chemical structures of compounds were elucidated by NMR and MS analysis. Among them, four compounds were isolated from the family Apocynaceae; two compounds were separated from the genus *Cynanchum*; and six compounds obtained from *C. auriculatum* (Xu et al., 2023).

C. auriculatum is a medicinal plant and it serves as edible food, has been cultivated for centuries. The enormous amounts of flavonoids, lignin, and other nutrients were present in tuberous roots of *C. auriculatum*. The analysis of phytohormone content and gene expression

revealed that auxin and cytokinin play a key role in development of tuberous root, while the biosynthesis and signalling of ethylene were inhibited. When the flavonoid increase, synthesis genes was also up-regulated. The flavonoid synthesis pathway was enhanced by reducing the activity of key enzymes of lignin synthesis in *C. auriculatum* (Sun et al., 2023).

Li et al. studied the five new cynotogenins (C_{21}-steroidal sapogenins) were isolated from the acid hydrolysate of *Cynanchum otophyllum* roots. Their structures were elucidated by using spectroscopic analysis such as UV, IR, HR-ESI-MS and NMR. Particularly, 5β, 6β-epoxy group present in the C_{21}-steroidal skeleton of *Cynanchum* plants. All compounds were evaluated for their cytotoxicity against multiple cancer cell lines, which exhibited more cytotoxic activity (Li et al., 2023).

The roots of *Cynanchum otophyllum* belongs to Apocynaceae family, commonly known as Qingyangshen in Chinese, were used as traditional medicine by different ethnic communities for longer period. It is used to treat epilepsy, rheumatism and inflammatory diseases. It can also use in cosmetics and food supplement (detox products), and in phyto-preparations used to reduce hair loss. The plant is a rich reservoir of C21 steroidal glycosides. Many bioactive compounds have been isolated and some of them have been pharmacologically characterized, such as otophyllosides, cynotophyllosides, cynanotins, cynotogenins, cynanchins. *C. otophyllum* contain proteases which are exploited for the local preparation of a cheese-like milk cake (Bailly et al., 2023).

Cynanchum paniculatum has been applied for various diseases especially the polysaccharide components extracted by ethanol. The bioactivities experiment results suggested that the polysaccharide exhibited strong potential of antioxidant activities and antitumor effects in both *in vitro* and *in vivo*. The *C. paniculatum* ethanol extracted polysaccharide hold a great application, novel bioactive component due to the feasibility of industrial production (Ji et al., 2022).

In vitro seed culture is the most successful method of conserving a rare, threatened, endangered, and vulnerable medicinal plants. *Cynanchum tunicatum* is a climbing shrub native to India and Sri Lanka. The plant seeds are used in traditional medicine to treat fever, skin diseases and infections. Producing ample plantlets through the seed culture technique is a boon and overcoming the exploitation of medicinal plants to optimize the experimental factors using Response Surface Methodology (RSM) 2FI Model. Since the significance of abiotic factors plays an importance role in plant tissue culture. Hence, RSM was analysed to optimize the

influencing factors such as pH, photoperiod and sucrose concentration on Murashige and Skoog (MS) basal medium. The factors were optimized under various experimental conditions, the maximum percentage (96%) of seed germination was obtained at pH 5.8, photoperiod 16/8 hours with 3% of sucrose (Krishnamoorthy et al., 2023).

Wang et al. examined medium salt strength and sucrose concentration which affected adventitious roots (AR) biomass and bioactive compound accumulation of *C. wilfordii*. The MS medium was most beneficial for increasing AR biomass and contents of polysaccharides, flavonoids, and acetophenone derivatives (ρ-hydroxyacetophenone, 2,5-dihydroxyacetophenone, and 2,4-dihydroxyacetophenone. Among the sucrose concentrations (10 to 70 g L^{-1}), 30 g L^{-1} sucrose increased AR biomass and flavonoid, ρ-hydroxyacetophenone, and 2,5-dihydroxyacetophenone accumulation, but 50 and 70 g L^{-1} sucrose was better for 2,4-dihydroxyacetophenone and polysaccharide accumulation, respectively. The kinetic study indicated that the culture period of 25 days was the most favourable for AR biomass and acetophenone derivative accumulation, whereas 30 d was beneficial for polysaccharide and flavonoid accumulation. The comparison study revealed that a large bioreactor size (5 to 20 L) is beneficial for the biomass and bioactive compound accumulation, indicated good applicability of the AR biomass established in a 5-L bioreactor (Wang et al., 2023).

According to Hamidi et al. the plant *Cynanchum acutum* has the possibility to produce the specific herbicide. This plant is rich in biologically active compounds, so it can be valued and exploited to produce specific biopesticides. The aqueous extract of the plant contained coumarins, saponins polyphenols, flavonoids, alcaloids, terpenes, tannins, quinones, aldehydes, and cardioglycoside. The inhibition process was confirmed by the microscopic study to detect the cytotoxicity of the seeds of *Arachis hypogaea*. Treatment with an aqueous extract that inhibit root growth (50%), which showed a decrease in the mitotic index with the observation of chromosomal abnormalities such as the beginning of the formation of 2 micronucleus at interphase, binucleated cells at interphase, disturbed at metaphase, stickiness at metaphase, oblique at metaphase, fragments at metaphase, bridge at anaphase, and binucleated cells diagonal (Hamidi et al., 2023).

The method for simultaneous determination of the two types of principal components, C21- steroids and acetophenones of *Cynanchum bungei*, *C. auriculatum* and *C. wilfordii* by HPLC-UV was developed. Under the optimized conditions, good linearities ($R2 \geq 0.999$) were obtained for all analytes, and relative standard deviations of HPLC-UV method validation

ranged from 0.01-1.62%. The DPPH and ABTS free radical scavenging assays indicated that the three species had a significant antioxidant activity, with EC_{50} range of 64.56-593.38 μg/mL. Combined with bivariate analysis, the finger print activity relationship of the offline antioxidant activity of the three species with their fingerprints peak, and the results revealed a dose-effect relationship between acetophenones and antioxidant activity (He et al., 2023).

Shu et al. studied the two new rhamnosides, 18-O-*α*-L-rhamnopyranosylabietic acid and *(E)*-3,5-dimethoxystilben-4′-O-*α*-L-rhamnopyranoside, five known glucosides along with three others were isolated from *Cynanchum atratum* roots. The structures of new compounds were elucidated by physical data analyses such as NMR, UV, IR, HR-ESI-MS, as well as acid hydrolysis. All of them were assessed for their antioxidant activities through $ABTS^{\bullet+}$, DPPH and PTIO assay. These bioactive components could be promising antioxidants (Shu et al., 2023).

Ding et al. investigated the chemical constituents and chemotaxonomic significance from the flowers of *Cynanchum auriculatum* by phytochemical methods. Sixteen compounds were identified including phenols, flavonoids and others were yielded. Their chemical structures were elucidated by NMR and MS analysis. Notably, seven compounds were isolated from Apocynaceae; while five compounds were reported from *C. auriculatum* (Ding et al., 2023).

The analgesic effect and material basis of *Cynanchum komarovii* were determined. The analgesic effect of TACKI and provide experimental data support for its traditional application in the treatment of various pains. Acute toxicity experiments showed that LD_{50} of TACKI mice was 2960.88 mg/kg, and symptoms of poisoning appeared. Pathology of liver and kidney studies have shown that TACKI reduced eosinophils and increases basophils in kidney glomeruli. TACKI had analgesic activity through the PWL and formalin test. In the chronic inflammatory antinociceptive activity, the latency of the withdrawal reflex in the TACKI group was prolonged, and the mechanical withdrawal reflex threshold was significantly increased. The protein expression of NMDA, GFAP and Iba-1 in rat brain tissue can be reduced significantly by TACKI. Meanwhile, the content of TNF-*α* and IL-6 in rat brain tissue is reduced (Wang et al., 2022).

Bungarus multicinctus is one of the top ten venomous snakes in China. Its venom is mainly neurotoxin. The molecular mechanism of *Cynanchum paniculatum* were treated with *B. multicinctus* bites based on network pharmacology. The potential active ingredients of *C. paniculatum* were screened and their SDF structures were obtained using PubChem database

and modelling with help of Swiss Target Prediction database. The targets were obtained for the antitoxin effects of *C. paniculatum* in the treatment of *B. multicinctus* bites. The *C. paniculatum* active compound were potentially target network and protein-protein interaction network were constructed by Cytoscape software. The biological function analysis and KEGG pathway enrichment analysis were performed using DAVID. Seven potential active components such as cynapanoside C, cynatratoside B, tomentolide A, sitosterol, sarcostin, tomentogenin, paeonol and 286 drug targets were obtained, including 30 key targets for the treatment of bungarotoxin toxicity. The active components mainly acted on PIK3CA, MAPK1, MAP2K1, JAK2, FYN, ACHE, CHRNA7, CHRNA4, and CHRNB2, and they antagonized the inhibitory effect of bungarotoxin on the nervous system through cholinergic synapses and the neurotrophin signaling pathway. It exerts a therapeutic effect on *B. multicinctus* bites through multiple active components and pathways (Zeng et al., 2022).

A water-soluble neutral polysaccharide (CAPW-1) with an average molecular weight of 64 kDa was purified from *Cynanchum atratum* root. The monosaccharide residue analysis revealed that CAPW-1 was composed of arabinose and galactose with a relative molar ratio of 7: 3. The backbone of CAPW-1 was consisted of 1,3-Gal*p* and 1,3,6-Gal*p*, the branches were attached to the O-6 of 1,3-Gal*p*, and the side chains contained 1,6-Gal*p*, 1,3,6-Gal*p*, 1,5-linked, 1,3-linked, 1,3,5-linked, and terminal-Ara*f*, which was attached to the O-3 of side 1,6-Gal*p*. The bioactivity indicated CAPW-1 could stimulate the proliferation of RAW264.7 cells and promote the secretion of nitric oxide (NO), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) with no cytotoxicity. It suggested a potential application of CAPW-1 as an immunostimulant for the treatment of diseases such as infection and tumour (Li et al., 2022).

Cynanchum rostellatum is a perennial herbaceous twining vine which is widely distributed in Japan, South Korea, the United States of America, and China. The complete chloroplast (cp) genome of *C. rostellatum* was sequenced using the Illumina platform and assembled. The plastome has a circular structure with a length of 160,641 bp. The GC content of the plastome was 37.82%. The cp genome contained 113 unique genes, including 79 protein-coding, 30 transfer RNA, and four ribosomal RNA genes. Phylogenetic analysis based on the complete cp genome sequences showed *C. rostellatum* was closely related to *C. bungei* (Pei et al., 2022).

The distribution patterns of invasive weed species under climate change are critical for the early identification from vulnerable regions. *C. acutum* were existing in certain regions and

predicted under various climate scenarios, using maximum entropy algorithm. Species occurrence data were represented the natural distribution of *C. acutum* and 15 of the World Clim bioclimatic variables. With an ensemble method, the impact of climate change on distribution of species is predicted according to five CMIP6 climate change models and three scenarios. *C. acutum* could expand its range to the north, particularly in agricultural landscapes. This emphasized the need to determine the priority of conservation targets, especially for agricultural areas, to ensure food safety and protect biodiversity (Ar et al., 2022).

The anti-diabetic compounds with effective suppression activities against hepatic glucagon response were required for the development of new drugs against diabetes. Well known fungi used for their ability to produce new bioactive secondary metabolites. Indole-terpenoids, named encindolenes were isolated from fungus *Penicillium* sp. HFF16 from rhizosphere soil of *C. bungei*. The structures of the compounds were elucidated by spectroscopic data and ECD analysis. In anti-diabetic activity assay, compounds could inhibit the hepatic glucose production with EC_{50} values of 17.6, 30.1, 21.3, 9.6, and 9.9 μM and decrease the cAMP contents in glucagon-induced HepG2 cells (Xiao et al., 2022).

A pregnane C21-steroid, cynansteroid F and 20-hydroxylatecaudatin, together with seven known compounds were isolated from roots of *C. auriculatum*. The chemical structures were elucidated by analysis of physiochemical properties, 1D-NMR, 2D-NMR, HRESI-MS, IR and ECD spectral data. Among them, eight compounds were isolated from the roots of *C. auriculatum* (Huang et al., 2022).

Cynanchum taiwanianum is an important plant used in traditional medicine. The effects of nitrogen and potassium fertilization rates on growth and biosynthesis of main bioactive compounds, including cynandione A and polyphenolic compounds from *C. taiwanianum* were examined. Two field experiments were analysed using three levels of nitrogen (N100, N150 and N200) and three levels of potassium (K100, K150 and K200) treatments. The experimental variables were either N or K fertilizer. Aside from N200, N and K fertilization significantly increased from *C. taiwanianum* shoot and tuber biomass. High N fertilization resulted in low total phenolic and flavonoid contents in shoots and tubers, but the effects of K fertilization were minimal. Cynandione A is an important bioactive compound, was only detected in tubers; its content was enhanced with increasing K fertilization, but reduced with excess N fertilization (N200). Although N and K fertilizers are important for *C. taiwanianum* tuber production, the yield of cynandione A was associated with K but not N fertilization rates (Tseng et al., 2022).

Traditional Chinese medicine has been frequently used as skin lightning agents. However, the mechanism of action of their effect is unclear. The evaluation of anti-tyrosinase activity of 10 commonly used TCM on mushroom (ab), human (hs) and mouse melanoma B16F0 (mm) tyrosinase (TYR). At 1.0 mg/mL, extracts from *Rosa rugosa*, *Morus alba* and *Paeonia lactiflora* were active against both abTYR and hsTYR (>50% inhibition), extracts from *Bletilla striata*, *Centella asiatica*, *Cynanchum atratum*, *Rosa canina*, *Rhus chinensis* and *Glycyrrhiza urolensis* inhibited either abTYR or hsTYR (>50%), while extract from *Tribulus terrestris* had no minimal activity (Liu et al., 2022).

Cynotofuranoside and C21-steroidal furanosides were isolated from acid hydrolysate of *Cynanchum otophyllum* roots. Their structures were elucidated by extensive spectroscopic analysis including UV, IR, 1D and 2D NMR and HR-ESI-MS. Compounds represented the 2,6-dideoxy-aldohexose in the form of furan ring attaching to 3β-OH of natural occurring C21-steroidal aglycones. The cytotoxicity of these compounds against human cancer MCF-7, HCT-116, HeLa, and HepG2 cells were evaluated. Cynotofuranoside and C21-steroidal furanosides showed moderate cytotoxic activities against HepG2 cancer cells with IC_{50} values of 43.15 and 34.36 μM respectively (Li et al., 2022).

Developing the high-efficiency and low-risk small-molecule green nematocide is effective to control the nematodes. Paeonol is a naturally occurring phenolic compound, isolated from root bark of *Paeonia suffruticosa* and the whole plant of *Cynanchum paniculatum*. Due to its crucial phenolic ketone skeleton, modern biological science research has indicated that paeonol has a wide range of biological activities. The biorational natural products-based pesticidal agents, 4 significant intermediates and 21 novel 3/5(3,5)-(di)nitro/chloropaeonol carbonyl hydrazone derivatives were prepared, and their structures well characterized by ^{1}H NMR, HRMS and MS. Due to the steric hindrance, the substituents on the C=N double bond of all hydrazine compounds adopted E configuration. Among all compounds, especially 5-nitropaeonol and 3,5-dinitropaeonol displayed the most potent nematicidal activity of *H. glycines* in *in vivo* with LC_{50} values of 0.0323 and 0.0367 mg/mL. It suggested that the 3/5(3,5)-(di)nitro/chloropaeonol carbonyl hydrazone derivatives, a nitro group introduced at C_5 position of potent compound as nematicidal agents (Chen et al., 2022).

Sub-chronic toxicity studies using rats were analysed from *Cynanchum wilfordii* and *Cynanchum auriculatum*. *C. wilfordii* water extract didn't show any adverse effects whereas administering *C. wilfordii* powder decreased body weights in complication with decreased food

consumptions. In the case of *C. auriculatum* water extract, triglyceride, absolute liver weights were elevated and vacuolation was observed in liver. Treated *C. auriculatum* powder in male rats increased alanine aminotransferase, aspartate aminotransferase, induced single cell necrosis and multinucleated hepatocyte in liver. As for female rats, increased absolute weights, vacuolation in adrenal glands and vacuolation in ovaries were observed when administered *C. auriculatum* powder. There is no observed adverse effect level (NOAEL) of *C. wilfordii* water extract was over 5000 mg/kg/day, while NOAEL of *C. wilfordii* powder was 700 mg/kg/day for female and 150 mg/kg/day for male. In case of *C. auriculatum*, NOAEL of water extract was 1500 mg/kg/day for male and 2000 mg/kg/day for female, while NOAEL of powder was 150 mg/kg/day for both gender (Yu et al., 2022).

Cynanchum auriculatum is a kind of critical Chinese herbal medicine. Deterioration of the natural environment severely affected the growth and development of *C. auriculatum*. The screening and identifying the suitable reference genes of *C. auriculatum* under various stress conditions. Based on qRT-PCR, geNorm, NormFinder, BestKeeper, and RefFinder were used for the expression stability evaluation of 12 potential reference genes from *C. auriculatum*. The ranking table showed that optimal reference genes included *EF2* and *SAMDC* (heat stress), *CYP* and *TUB-β* (coldstress), *TUB-α* and *GAPDH* (drought stress), *SAMDC* and *TUB-α* (waterlogging stress), along with *EF2* and *ACT7* (salt stress) (Zhu et al., 2022).

Six phenolic compounds such as talaroflavone, alternarienoic acid, altenuene, altenusin, alternariol, and alternariol-5-*O*-methyl ether were isolated from the solid rice culture media of *Alternaria* sp., an endophyte isolated from fresh leaves of three desert plants, *Lycium schweinfurthii*, *Pancratium maritimum* and *Cynanchum acutum*. Alternarienoic acid, altenuene, altenusin exhibited potent α-glucosidase and lipase inhibitory activities suggesting that they might act as naturally occurring anti-diabetic candidates. The same compounds showed potent binding in the active site for both enzymes with desirable pharmacokinetic properties. The isolated bioactive compounds were not exclusive to a certain host plant which revealed the dominant ecological standpoints for consequent optimization. This could lead to a cost-effective and reproducible yield to commercial scale-up (Elbermawi et al., 2022).

Atopic dermatitis (AD) is a chronic inflammatory skin disease accompanied by severe itching and dry skin. Cynanoside F (CF) is one of the pregnane-type compounds from *Cynanchum atratum* root, an oriental medicinal herb that has been shown to have antioxidant, antitumor, and anti-inflammatory effects. Although CF has been isolated as a component in

Cynanchum atratum, the scientific role of CF has not yet been explored. The effect of CF on AD and revealed the mechanism using *in vitro* and *in vivo* experimental models. CF significantly reduced lipopolysaccharide (LPS)-induced protein expression levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2), which are important proinflammatory mediators in the RAW264.7 macrophage cell line. CF did not inhibit the nuclear factor-kappa B (NF-κB) signaling activated by LPS but significantly reduced the phosphorylation of mitogen-activated protein kinases (MAPKs), such as p38 MAPK, JNK, and ERK. CF consistently inhibited the activity of the activator protein-1 (AP-1) transcription factor, a downstream molecule of MAPK signaling. In an experiment using an oxazolone-induced AD mouse model, the CF-treated group showed a marked decrease in epidermal thickness, the number of infiltrated mast cells, and the amount of histamine. The mRNA levels of IL-1β, interleukin-4 (IL-4), and thymic stromal lymphopoietin (TSLP) were consistently lowered in the group treated with CF. Moreover, the phosphorylation of c-Jun and c-Fos protein levels, which are the AP-1 components, were lowered in the skin tissues of CF-treated mice (Fleitas et al., 2022).

The optimal medium composition for the growth of *Cynanchum wilfordii* adventious roots and the content of central primary and bioactive specialized metabolites for the efficient selection of mother plants. It found that 3/4 MS salt medium, 4.92 μM IBA, and 5% sucrose were the best for the proliferation of ARs originating from cultivated on a heap (ECH) and open field (ECF). Multivariate analysis showed the metabolite differences and correlations to be derived from common or closely related pathways related to abiotic stress. ARs from ECH featured higher levels of phenolic compounds, polysaccharides, aromatic amino acids, hydroxyacetophenones, and mono-unsaturated fatty acids, which were associated with various mechanisms to protect the mother plants from abiotic stress. ECH is a better line for the production of valuable specialized metabolites that participate in the biosynthesis of flavonoids and hydroxyacetophenones, including rutin, epicatechin, kaempferol, and 4′-hydroxyacetophenone. It suggested that substrates to increase the content of functional specialized metabolites (Hyeon et al., 2022).

In vitro propagation is an effective method for the conservation and development of the *Jasminanthes tuyetanhiae* plantlets belongs to Apocyanaceae on a commercial scale. The surface sterilization method with $HgCl_2$ is effective for primary *in vitro* explant generation, BA and IBA are plant growth regulators that at optimum concentrations have improved *in vitro* rapid multiplication of shoot and root. A nylon bag culture system with a millipore membrane

that is ventilated can be considered as a useful system for increasing the growth and development of *J. tuyetanhiae* (Nam et al., 2022).

In *Cynanchum acutum,* the silver nanoparticle synthesis from latex and its crude latex on biochemical, molecular DNA level and mitotic division using *Vicia faba* seeds. The reducing effect of the MI% was clearly observed by increasing Cy-AgNP concentrations (50 and 100 mg/L, where Cy-AgNPs (25 mg/L) treatment showed moderate decrease in MI% compared to *C. acutum* latex (3%). Generally, all treatments showed increasing chromosomal abnormalities, but Cy-AgNPs (25 mg/L) expressed the lowest percentage, and by increasing the concentration of AgNPs, the percentage increased. Genomic template stability percentage (GTS%) using biochemical protein SDS-PAGE and molecular ISSR markers showed the highest GTS% in the 25 mg/L Cy-AgNPs treatment (80% in SDS-PAGE and 51.28% in ISSR marker). The 25 mg/L Cy-AgNPs have the highest content of bioactive constituents (TPC, TFC, tannins, and alkaloids) and showed lowest cytotoxicity and genotoxicity. The highest antioxidant activity using the DPPH method was reported in Cy-AgNPs (25 mg/L) (70.26 ± 1.32%). The highest antibacterial activity was for Cy-AgNPs (50 mg/L) against *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus*. Chemical characterization of GC-MS revealed that n-alkanes such as tetradecanoic acid and hexadecanoic acid had the highest antimicrobial effect in addition to the presence of lupeol effect on the antioxidant activity (Soliman et al., 2022).

The phytochemical screening reported C21 steroidal glycosides rich in the root extract of *Cynanchum auriculatum* is a traditional Chinese medicine. The isolation of 27 C21 steroidal glycosides from the root extract which possessed antioxidant effect. It was evaluated using the H_2O_2-treated PC21 cells. All the tested compounds altered the activities of lactate dehydrogenase, superoxide dismutase, catalase and glutathione peroxidase at concentrations as low as 1 μM in H_2O_2-treated PC21 cells. They also decreased the levels of intracellular reactive oxygen species and Ca^{2+}. Further, the correlation between their structural features described by molecular descriptors and the indicators of bioactivity was analysed by partial least squares analysis, displaying those six bioindicators were positive correlated with 13 molecular descriptors. (Zhang et al., 2022).

Shu et al. isolated a novel monoterpene rhamnoside (1) and 7 known monoterpenes (2-8) from ethanol extract of *Cynanchum atratum*. Their structures were identified by comprehensive spectroscopic data analysis such as nuclear magnetic resonance, high-resolution electrospray ionization mass spectra, optical rotatory dispersion, and acid

hydrolysis. In the subsequent antioxidant assay, compound 8 exhibited 2,2-diphenyl-2-picrylhydrazyl hydrate radical scavenging activity (Shu et al., 2022).

Six unidentified C21 steroidal glycosides, cynwallosides A−F, as well as twenty-two known compounds were isolated from roots of *Cynanchum wallichii*. The structures of cynwallosides A−F were determined by spectroscopic analysis and acidic hydrolysis. Most of these twenty-eight compounds were found to significantly reverse drug resistance in both the MCF-7/ADR and HepG2/ADM cell lines by suppressing P-gp protein expression (Zhang et al., 2022).

Wang et al. studied the potential biologically active and structurally unique steroidal glycosides, phytochemical investigation of *Cynanchum taihangense*. Twelve new seco-pregnane glycosides, cynataihosides, and two known glycosides, glaucoside A and atratcynoside F were isolated from the 95% ethanol extract of *C. taihangense*. The structures of the glycosides were elucidated based on 1D and 2D NMR spectroscopic data, HR-ESI-MS analysis. The cytotoxicity of compounds against three human tumor cell lines (HL-60, THP-1, and PC-3) were evaluated by MTT assay. The compound displayed significant cytotoxicity against THP-1 and PC-3 cell line with IC_{50} values of 5.08 and 22.75 μm, respectively. Compounds 3 and 14 exhibited moderate cytotoxicity on HL-60 and THP-1 with IC_{50} values of 17.78 and 16.02 μm, respectively (Y.-B. Wang et al., 2022).

Li et al. reported *Cynanchum otophyllum* which have C_{21}-steroidal aglycones exhibit antitumor effects. To get large scale C_{21}-steroidal aglycones, the extracts of roots were treated with 5% HCl in aqueous was investigated. Nine new C_{21}-steroidal aglycones (1–9) namely cynotogenins A-I, along with seventeen known analogous (10–26), were isolated from the hydrolysate. The structures of compounds 1–9 was elucidated by spectroscopic analysis (IR, HR-ESI-MS, 1D and 2D NMR) and compared with reference date. Aglycones 2–5 with rare *cis*-cinnamoyl group as well as 8 and 9 with 5β,6β-epoxy group were found from the genus of *Cynanchum*. Moreover, compound 20 inhibits HepG2 cell apoptosis and induced of G0/G1 phase which arrest in a dose dependent manner (Li et al., 2021).

Naturally occurring C21 steroidal glycosides have been isolated from *Cynanchum* plants. Mostly it was derived from the aglycone caudatin (CDT) which includes a tetracyclic deacylmetaplexigenin unit and an ikemaoyl ester side chain. CDT can be found in diverse traditional medicines, such as Baishouwu radix used to treat gastro-intestinal disorders and potential anticancer properties. CDT and its mono-glycoside analogue CDMC display antiproliferative activities against different cancer cell lines and revealed significant anticancer effects in tumor xenograft models in *in vivo*. Their mechanism of action is multifactorial,

implicating several signaling pathways (Wnt/GSK3/β-catenin, TRAIL/DR5/ER and TNFAIP1/NFκB) which contribute to the antiproliferative, antiangiogenic, antimetastatic and proapoptotic effects of the natural products. CDT modulated DNA replication, antioxidant and targets some cancer stem cells. CDT and CDMC are significant anticancer products, while other CDT glycoside derivatives displayed antiviral and antifungal activities (Bailly, 2021).

There are seven compounds were isolated from methanolic extract of *Cynanchum acutum*. The isolated compounds include six flavonoid compounds identified as rutin, quercetin-3-*O*-neohesperidoside, quercetin-3-*O*-*β*-galactoside, isoquercitrin, quercetin, and kaempferol 3-*O*-*β*-glucoside, coumarin, scopoletin. All tested compounds significantly reduced oxidative stress and increased erythrocyte lysate levels of antioxidant enzymes, along with the amelioration of the serum levels of inflammatory markers. Molecular modeling approach on two biological targets of NF-κB pathway managed to highlight the superior anti-inflammatory activity of quercetin-3-*O*-galactoside and quercetin as compared to other bioactive metabolites (Abdelhameed et al., 2021).

Cynanchum auriculatum, Cynanchum bungei and *Cynanchum wilfordii* are three close species belongs to Asclepiadaceous family. Their dry roots contain bioactive compounds to exhibit anti-tumor, neuroprotection, organ protection, reducing liver lipid and blood lipid, immunomodulatory, anti-inflammatory, and other activities. The phytochemical investigation revealed 232 compounds from these three species which could be classified into C_{21}-steroids, acetophenones, terpenoids, and alkaloids (Wang et al., 2021).

C. atratum is a traditional Chinese medicinal plant used to treat various ailments. The plant tissue culture technology has been used to establish a fast and efficient propagation system through axillary bud proliferation. The MS medium supplemented with NAA 0.2 mg L^{-1}+IBA 1.5mg L^{-1}+Kin 0.5 mg L^{-1} is effective in proliferating adventitious buds with a 100% induction rate and 8.56 proliferation coefficient. MS medium induced adventitious bud rooting with a 98% success rate. The best survival rate (90%) was achieved with a grass mud pond and orchard red soil ratio of 1:1 (Chang et al., 2021).

Abdelhameed et al. utilized the various chromatographic techniques led to the isolation of seven compounds from *C. acutum* plant extract, including a coumarin and six flavonoid compounds. The antioxidant and anti-inflammatory properties of five of the isolated flavonoids such as quercetin-3-O-β-galactoside, quercetin, rutin, quercetin-3-O-neohesperdoside and isoquercitrin were assessed in a rat model of T2DM. All the investigated compounds

ameliorated the oxidative stress, reduced the levels of inflammatory markers, and regulated the expression of NF-κB and miR-146a in both liver and adipose tissue. The molecular docking simulation on two significant NF-κB targets; RelB: p52 heterodimer and hIKK, have predicted the binding preferentiality for quercetin-3-O-β-galactoside, even as compared to the crystallized ligand at the hIKK protein target. Furthermore, the docking investigation highlighted the molecular promiscuity of the isolated flavonoids, allowing them to modulate multiple molecular pathways and targets. Conformation of the obtained *in silico* study were carried out through experimental studies, testing the effect of compounds on NF-κB, by assessing the phosphorylation and total levels of p65 and IKK by Western blotting (Abdelhameed et al., 2021).

In *Cynanchum limprichtii* seventeen compounds were isolated and characterized which have been obtained from phytochemical investigation from the root extract. It included eight steroidal glycosides, one flavone, three aromatic compounds, four organic acidic compounds, and one sugar. Their structures were established by spectroscopic methods, which were compared to reference data (Liu et al., 2021).

Soma is an important Ayurvedic drug used for various therapeutic aspects. *Cynanchum viminale* and *Ceropegia juncea* are being used as two important source plants of Soma. Two plants were compared phytochemically using various quantitative assays and chromatographic techniques like HPTLC and HPLC. On quantitative assays *C. viminale* showed higher flavonoid content whereas *C. juncea* showed higher percentage of phenolics, carbohydrates and amino acid contents. The presence of terpenoid and steroid was identified in plants using HPTLC. Both plants showed similar steroids and terpenoids profile. Detailed chromatographic analysis was revealed that source plants of Somalata such as *C. viminale* and *C. juncea* are almost similar in their phytochemical profiles (Deepak et al., 2021).

Four new indole-terpenoids (1–4) named encindolene A, 18-*O*-methyl-encindolene A, encindolene B, and encindolene C, as well as three known analogs (5–7), were isolated from the fungus *Penicillium* sp. HFF16 from the rhizosphere soil of *Cynanchum bungei*. The structures of compounds including absolute configurations were elucidated by spectroscopic data and electronic circular dichroism (ECD) analysis. Anti-inflammatory activity evaluation revealed that compounds 1–7 inhibit the production of nitric oxide with IC_{50} values of 79.4, 49.7, 81.3, 40.2, 86.7, 90.1, and 54.4 μM, respectively, and decrease the levels of tumor necrosis factor-α, interleukin-6 contents in lipopolysaccharide-induced RAW264.7 macrophages (Pan et al., 2021).

Cynanchum paniculatum (CP) is an important medicinal herb used in Chinese herbal medicine, with a variety of biological activities including anticancer property. The aqueous extract of CP, for its anticancer effects against breast cancer cells with different mutation types. Cells were grouped as untreated; CP direct treatment (dir-CP); Conditioned medium from CP treated (sup-CP), and untreated cells (sup-Control). Effects of dir-CP and sup-CP were compared to corresponding untreated cells on cytotoxicity, cell migration, and protein expression (cleaved caspase-3, caspase-9, and MMP-2 and 9). CP treatment showed time-dependent decrease in cell number of MDA-MB-231 and SK-Br-3 (both ER (−) PR(−)), while the decrease in cell number was not as significant in MCF-7 and ZR-75-1 cells (both ER(+) PR(+)). sup-CP treatment inhibited the cell migration of MDA-MB-231 and MCF-7 (Her2(−)) in a 24 h scratch assay. The ER(−) PR(−) cells are more sensitive to the CP in terms of direct cytotoxicity, which is not regulated by caspase-3. CP inhibited the migration of the two Her2(−) cells, and this correlated with MMP-2 regulation. The migration of ER(−) PR(−) cells was more sensitive to conditioned medium with CP treatment than to direct CP, and this is not regulated by MMP-2. CP has anticancer potential on various breast cancer cells through different mechanisms and is specifically effective in inhibiting the migration of the triple negative MDA-MB-231. The mechanism of CP against breast cancer progression and would benefit the medical practitioners (Yang et al., 2021).

Cynanchum thesioides is a member of the *Asclepiadaceae* family that have been used in traditional Chinese medicine and their latex contains succinic acid which has an important medicinal value. To identify candidate genes involved in succinic acid biosynthesis and accumulation, the transcriptome data from *C. thesioides* under drought stress using *Illumina* sequencing technology. A total of 146 and 172 unique genes were annotated as encoding enzymes involved in the succinic acid biosynthesis of *C. thesioides* and *C. thesioides* var. australe and number of differentially expressed genes (DEGs) was 40 and 32, respectively. As drought stress increased, nine genes were significantly up-regulated and seven genes were significantly down-regulated in *C. thesioides*. In *C. thesioides* var. australe seven genes were up-regulated and nine were down-regulated. Enzymes participating in succinic acid biosynthesis were ATP citrate (pro-S)-lyase, succinate dehydrogenase, aconitate hydratase, 2-oxoglutarate dehydrogenase, citrate synthase, glutamate synthase, fumarate hydratase, succinyl-CoA synthetase, succinate-semialdehyde dehydrogenase, glutamate dehydrogenase, isocitrate dehydrogenase, 4-aminobutyrate transaminase, malate dehydrogenase, and glutamate decarboxylase and their activities relating to succinic acid synthesis were determined. RNA-seq sequencing was used. The genes involved in biosynthesis and accumulation of succinic

acid in *C. thesioides* were predicted. Succinic acid content was positively correlated with *MDH* (BMK_Unigene_033366), but negatively correlated with *GAD* (BMK_Unigene_096180 and BMK_Unigene_030468), *SDH* (BMK_Unigene_142841) (Zhang et al., 2021).

The antidiabetic potential of pregnanes from Apocynaceae, sixteen derivatives 1-1 were isolated from various *Cynanchum* species have been evaluated for their ability to increase glucagon-like peptide-1 (GLP-1) release in a cell assay. The otophylloside A (2), wilfoside C1N (13), and wilfoside C3N (16) were found to stimulate significantly the secretion of GLP-1, doubling the extracellular content of this incretin. Caudatin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside (6) was also bioactive, but to a lesser extent (133 % of secretion compared to control cells). The potential of certain pregnane derivatives from Asclepiadoideae to stimulate the release of GLP-1, reduced in patients with type 2 diabetes (Huguet et al., 2021).

Cynanchum thesioides is an herb of traditional Mongolian medicine that has been employed in treating abdominal pain and diarrhoea for hundreds of years. Phytochemical studies showed the presence of various flavonoids with antibacterial and anti-inflammatory activities. The gut mycobiome and reverses visceral hypersensitivity in IBS rat model. Treatment with *C. thesioides* improved visceral hypersensitivity in MS rats, and this was accompanied by alterations in the structure and composition of the gut microbiota. The extent of stability of the gut microbiota was improved after treatment with *C. thesioides*. The genera *Pseudomonas, Lachnospiracea incertae sedis,* and *Clostridium XlVa* (which were more prevalent in MS rats) were significantly decreased, whereas the abundance of some genera was less prevalent in MS rats, *Clostridium IV, Elusimicrobium, Clostridium sensu stricto,* and *Acetatifactor* were significantly enriched after treatment with *C. thesioides* (Lingpeng et al., 2021).

Cynanchum auriculatum is a traditional herbal medicine in China and can grow in saline soils. However, little is known in relation to the underlying molecular mechanisms. *C. auriculatum* seedlings were exposed to 3.75% and 7.5% salinity. Next, transcriptome profiles of leaves were compared. Transcriptome sequencing showed 35,593 and 58,046 differentially expressed genes (DEGs) in treatments with 3.75% and 7.5%, compared with the control, respectively. Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of these DEGs enriched various defense-related biological pathways, including ROS scavenging, ion transportation, lipid metabolism and plant hormone signaling. Further analyses suggested that *C. auriculatum* up-regulated Na^+/H^+ exchanger and V-type proton ATPase to avoid accumulation of Na^+. The flavonoid and phenylpropanoids biosynthesis pathways were

activated, which might increase antioxidant capacity in response to saline stress. The auxin and ethylene signaling pathways were upregulated in response to saline treatments, both of which are important plant hormones (Zhang et al., 2020).

Cynanchum otophyllum was traditionally used for treating muscle and skeletal pain, epilepsy, abdominal pain, and tightness. Four known C21 steroidal glycosides, namely caudatin 3-O-*β*-cymaropyranoside (1), caudatin 3-O-*β*-D-cymaropyranosyl-(1 → 4)-*β*-D-cymaropyranoside (2), otophylloside B (3), and otophylloside A (4) were isolated from the roots and their neurotrophic activities were examined in rat neuronal PC12 cell model, while their cytotoxicities were evaluated in human cancer cells and normal fibroblasts. Differential promoting effects on neurite-bearing cells were found among tested compounds, with 2 the most potent. Selective cytotoxicity against human colon cancer cells HCT-116 of 2. Besides, 2 induced inhibitory activities on cancer cell proliferation, sphere formation and interfered the cell cycle. The new potential pharmacological activities of 2 on colon cancer cells were detrined (Dong et al., 2020).

Kim et al. isolated a Cynandione A (CA) from ethyl acetate extract of *Cynanchum wilfordii*, is a bioactive phytochemical that has been found to be beneficial for the treatment of several diseases. Hepatic *de novo* lipogenesis is one of the main causes of non-alcoholic fatty liver disease (NAFLD), which is thought to be a hepatic manifestation of certain metabolic syndromes. The ethyl acetate extract of *C. wilfordii* decreased the mRNA levels of sterol regulatory element-binding protein-1c (SREBP-1c), which played a crucial role in hepatic lipogenesis. Also observed that CA could suppress the level of SREBP-1c, which was increased using two commercial LXRα agonists, GW3954 and T0901317. Moreover, the enzymes that act downstream of SREBP-1c were also inhibited by CA treatment. To understand the mechanism underlying this effect, the levels of phosphorylated AMP kinase (pAMPK) were measured after CA treatment. Therefore, CA might increase the pAMPK level by inducing phosphorylation of liver kinase B1 (LKB1), which can then convert AMPK to pAMPK (S. Kim et al., 2020).

According to Jayamani et al. *Cynanchum viminale* is used for various ailments particularly microbial infections in Sri Lanka. The antimicrobial assays were carried out by agar well diffusion method using crude extracts. Five different bacterial species and three different *Candida* species were screened. Streptomycin sulphate and Fungicon were used as positive control for bacterial and fungal species, respectively. The phytochemical analysis showed positive to tannins, saponins, phlobatannins, flavonoids, cardiac glycosides, alkaloids, terpenoids and steroids. Ethyl acetate extract showed highest activity against *Escherichia coli*

with diameter of inhibition zones 26.9 ± 1.4 mm. The anti-fungal activity showed the highest activity (19.03±0.34) against *Candida krusei* (Jayamani et al., 2020).

A chemical investigation of *Cynanchum mongolicum* identified 8 compounds. On the basis of spectroscopic data, they were determined to be 3 alkaloids and 5 sinapoyl esters. The inhibitory effects of the isolated compounds against four human tumour cell lines were evaluated *in vitro* by MTT assays, which revealed moderate inhibitory effects with IC_{50} values < 50 mM, in particularly, three antofine analogues have showed significant antitumor activities with IC_{50} values < 0.1 mM, which was obviously better than 5-fluorouracil and potential to be used as cancer drugs (Yang et al., 2020).

Zhou et al. (2020) isolated and identified over 150 compounds from *Cynanchum paniculatum*, including C_{21} steroids, volatile oils, carbohydrates and phenanthroindolizidine alkaloids. Extensive pharmacological activities of the extracts or compounds of *C. paniculatum in vivo* and *in vitro* were confirmed including anti-inflammatory, anti-nociceptive, sedative antiviral, antitumor, neuroprotective, treating snake bites, immunomodulatory, anti-radiation, vasodilatory, acaricidal potentials and anti-adipogenic activities (Zhou et al., 2020).

The breast cancer stem cells (BCSCs) using natural compounds is a novel potential therapeutic strategy for clinical cancer treatment. A mammosphere assay-guided isolation protocol including silica gel, a C18 column, gel filtration, and HPLC was used to isolate an inhibitory compound from *Cynanchum auriculatum* extracts. The isolated inhibitory compound was identified as caudatin. Caudatin inhibited breast cancer cell proliferation, mammosphere formation and tumor growth. It decreased the $CD44^{+}/CD24^{-}$ and aldehyde dehydrogenase^{+} cell proportions and the levels of c-Myc, Oct4, Sox2, and CD44. It induced ubiquitin (Ub)-dependent glucocorticoid receptor (GR) degradation and blocked subsequent Yes-associated protein (YAP) nuclear accumulation and target gene transcription signals in BCSCs. It showed that GR/YAP signaling pathway regulates BCSC formation and that caudatin may be potential chemopreventive agent that targets breast cancer cells and CSCs (Zhen et al., 2020).

The menopausal syndrome caused by rapid changes in hormone levels greatly influences the quality of life of women. Though hormone replacement therapy (HRT) is widely used to treat the menopausal syndrome, it exhibited many side effects, including the risk of thrombosis, cardiovascular diseases, and increased incidence of breast cancer. Thus, diversifying the interest for phytotherapy-based materials as alternatives to HRT. Here, isolated a crude polysaccharide fraction (CWPF) from *Cynanchum wilfordii* root that alleviated the

ovariectomy-induced uterine atrophy and bone loss without changes in plasma estradiol concentration in mice. Increased plasma levels of follicle-stimulating hormone (FSH), alkaline phosphatase (ALP), osteocalcin (OC) in ovariectomized mice were also reduced to normal levels by CWPF administration. The inhibitory effects of CWPF on menopausal symptoms were mediated by the estrogen receptor β (ER-β) specific activation, not ER-α were found. Moreover, CWPF treatment suppressed the phosphorylation of Akt, suggested that CWPF alleviates post-menopausal symptoms by regulating ER-β related Akt signaling pathway. The polysaccharides corresponding to CWPF among the water-soluble extracts of CW could be used as a beneficial herbal alternative for the development of therapeutic agents to prevent menopausal syndrome in women (Lee et al., 2020).

Testosterone deficiency syndrome (TDS), also known as late-onset hypogonadism is a clinical and biochemical syndrome associated with advanced age and characterized by deficient serum testosterone levels. The *Elaeagnus multiflora* fruit (EMF) and *Cynanchum wilfordii* (CW) have been used in traditional herbal medicine. The investigation of therapeutic effects of EMF and CW mixtures (at the ratios of 3:7, 5:5, and 7:3) on TDS using TM3 cells and aging male rats. EMF and CW (at the ratios of 3:7, 5:5, and 7:3) significantly increased testosterone levels in TM3 cells ($p < 0.05$). The rats were orally administered EMCW (EMF and CW mixed at the ratio of 3:7 50, 100 and 200 mg/kg/day) for 4 weeks consecutively. After 4 weeks of EMCW administration, latency time on the rotarod test, and serum testosterone and dehydroepiandrosterone sulfate levels were significantly increased ($p < 0.05$ and $p < 0.01$). Moreover, the levels of globulin-bound sex hormones were decreased in the EMCW-fed groups. However, prostate-specific antigen levels did not differ among the groups. It suggested that EMCW can be effectively used to alleviate TDS (Jung et al., 2020).

Cynanchum bungei and its related species *C. auriculatum* and *C. wilfordii* are well known Chinese herbal medicines known by Baishouwu, and have a beneficial effect on health. Endophytic fungi exist widely inside host plants and are important components of plant micro-ecosystems. The fungal diversity in *Cynanchum* genus were characterized and compared the endophytic fungal communities among different plant organs, species and localities of Baishouwu by a High-throughput 18S rDNA gene Illumina MiSeq sequencing technique. The main factor affecting the distribution and population structure of endophytes was plant organs, rather than the species and localities. Moreover, Rank abundance curves demonstrated that the richness and evenness of JTG was the highest, while the evenness of JTY was the lowest, the richness of JTJ was the lowest. Furthermore, the samples from three localities had relatively

similar fungal communities, as indicated by PCA and UPGMA analysis. The *Fusarium, Exophiala, Plectosphaerella, Botryosphaeria*, and *Filobasidium* were the predominant fungal genera in the seven samples, which might play an important role in the plant micro-ecosystems and were beneficial to their host plant. It contributed to understand the diversity of the endophyte microbiome of Baishouwu and may be beneficial to the rational development and utilization of the medicinal plants (Chen et al., 2020).

Anatomical and ultrastructure features of stem from *Cynanchum forskaolianum* were examined by light microscopy, scanning electron microscopy and transmission electron microscopy. The investigation of adaptational aspects that enable to cope with its stressful habitats. The stem has multiple morpho-anatomical adaptations of which low surface/volume ratio is an indication of voluminous capacity to store water. Thin boundary layer results in reduced heat-storage capacity. Papillose shape of epidermal cells together with thick epicuticular wax with thin cuticle reduces water loss. Sunken stomata with deep encryption as well as low stomatal frequency decrease transpiration and enhance water. The large substomatal cavities with intercellular spaces in chlorenchyma promote gas exchange and diffusion of CO_2. Ultrastructure of chlorenchymatous cells showed that chloroplasts have many plastoglobuli and low stacking of thylakoids, which corresponds to high light stress and leads to chloroplast protection against photoinhibition. Cortex cells have highly undulated walls that lend flexibility during drought. Ultrastructure of these walls revealed light packed fibrillar material, which can play a major role in flexibility, enable cells to easily lose and refill with water to support activity of adjacent chlorenchyma cells. Xylem characteristics increase safety of water transport with less vulnerability to cavitation. These anatomical and ultrastructure features of stem showed *C. forskaolianum* as a highly adaptable species in its dry habitats (Masrahi, 2020).

Cynanchum paniculatum is usually used as an herbal medicine for treating many diseases. Paeonol is the main active component, and its content is the key indicator for quality control of *C. paniculatum*. The development of a rapid, accurate and precise method for quantitation of paeonol of *C. paniculatum* using ^{1}H NMR spectra. The deuterated solvent of methanol-d4 enabled satisfactory separation of the signals to be integrated in ^{1}H NMR spectrum. H-6 (δ 7.78) of ^{1}H NMR spectrum of *C. paniculatum* was selected as the feature signal for quantitation, and trimesic acid (TMA) was selected as an internal standard. Validation of the quantitative method was performed in terms of linearity, specificity, repeatability and stability. The quantitative ^{1}H NMR (qHNMR) applied to determine the

content of paeonol of *C. paniculatum* and showed a wider linearity range than the reported quantitation of paeonol. The simple extraction of paeonol from *C. paniculatum* was rapid and will prompt the application of the developed method. The qHNMR represented a feasible alternative to HPLC-based methods for quantitation of paeonol of *C. paniculatum*, and it was suitable for the quality control (Li et al., 2020).

The flavonoids were extracted from *Cynanchum auriculatum* in Binhai by ultrasonic wave and organic solvent. The effects of ethanol concentration (60% - 100%), liquid-to-solid mass ratio (10 :1 - 50 :1), extraction temperature (30 - 70), extraction time (10-50 min), and sonication power (240-400 W) on flavonoid extraction were studied by the single factor test. The extraction technology was further optimized by orthogonal design. The ethanol concentration was the most prominent factor determining the extraction yield, followed by liquid-to-solid mass ratio, extraction time, and extraction temperature. The optimum parameters for flavonoids extraction were as follows: liquid-to-solid mass ratio 20 :1, ethanol concentration 90%, extraction temperature 40°C, and extraction time 30 min. The extraction yield of 3.95 mg/g was obtained under the optimized conditions (Sun et al., 2020).

The effect of a standardized *Cynanchum wilfordii* extract on the lipid profiles of individuals with elevated total cholesterol (T-Chol) using double-blind randomized placebo-controlled design. Ninety-six Korean individuals with elevated T-Chol level (200–240 mg/dL) were recruited and randomly allocated to groups that received VasH300 (300 mg CWE/day, n= 32), VasH600 (600 mg CWE/day, n= 32), or a placebo (n= 32) group. Primary outcomes included T-Chol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride, and safety (adverse events, biochemical parameters, and hematological parameters). Data were compared using a one-way analysis of variance followed by Duncan's *post-hoc* tests (among groups) and paired t tests (within groups). Values for T-Chol and LDL-cholesterol were significantly reduced in the VasH300 and groups (VasH300: 4.0 and 6.4%, respectively; VasH600; 3.8 and 5.8% respectively; both $p < 0.05$) compared with the placebo group and were not dose-dependent. VasH300 significantly improved the lipid profiles of individuals with elevated T-Chol without any serious side effects. Daily supplementation with VasH might be an alternative strategy with which to modify cholesterol-related parameters, especially in individuals with elevated T-Chol levels (Shin et al., 2020).

Anatomical section of roots of *Cynanchum chinense* grown in different habitats, but it did not affect its normal growth. Under the salty environment, the number of layers of *C.*

chinense root cortex cells are relatively small, and the cells are arranged more closely, which is beneficial to the plants to absorb water and nutrients in the soil. The soil physical and chemical properties of different habitats of *C. chinense* and the concentration of cultivable microorganisms in rhizosphere soil were compared and found that the obvious difference in root structure of *C. chinense* may be caused by the difference of soil soluble salt content and soil culturable microorganism concentration (Wang et al., 2020).

Dried tuberous roots of *Cynanchum wilfordii* are known to relieve menopause symptoms. However, the dried roots of *C. wilfordii* are morphologically similar to those of *C. auriculatum*, which makes it difficult to distinguish. The differences between the two species at the cytogenetic level based on chromosome structure and composition remain to be elucidated. For chromosome slides, the roots were fixed in 8-hydroxyquinoline, digested with enzyme mixture, and spread on slides. 5S and 45S rDNA were used as cytogenetic markers for the analysis of nuclear genomes. The chromosome number of the two species was 2n = 22, with a relatively short length, 1.13 μm - 4.24 μm and 1.00 μm - 3.42 μm with respect to each other. Both species represent one pair of 5S and 45S rDNA signal on chromosome 1, at the proximal region and peri-centromeric region, respectively (Kim et al., 2020).

Cynanchum auriculatum, Cynanchum bungei and *Cynanchum wilfordii* were collectively known as Baishouwu are valuable medicinal herbs with various pharmacological activities. The mechanisms of their beneficial bioactivities and the structure activity relationship of the constituents, as well as their potential synergistic and antagonistic effects. The proper toxicology evaluation is crucial to guarantee the safety, efficacy, and eligibility for medical uses (Chen et al., 2019).

The MTT assay was used to evaluate the cytotoxicity of the *Cynanchum paniculatum* fraction in MDBK cells. The EA fraction was found to have a CC_{50} level of 18.2 μg/mL, which was same as the effective level for antiviral effect. The functional fraction of ethyl acetate contains single compound which is responsible for the antiviral effect without any cytotoxicity (Kim et al., 2017).