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Review

Extraction, Separation, Antitumor Effect, and Mechanism of Alkaloids in *Sophora alopecuroides*: A Review

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Abstract: Malignant tumors pose a serious threat to human health, reducing quality of life. Natural antitumor drugs play a vital role in the treatment of cancer. *Sophora alopecuroides*, a traditional Chinese medicine not a part of the Chinese Pharmacopoeia, grows in the arid desert and edge zone of grassland. Previous studies have extensively investigated the antitumor effects of *S. alopecuroides* and its major alkaloids. Of these, aloperine, matrine, oxymatrine, sophoridine, and sophocarpine have received the most attention. In recent years, a variety of extraction and separation methods have been applied to the study of the alkaloids of *Sophora alopecuroides*, which has greatly promoted the study of the chemical constituents and pharmacological activities of the plant. *S. alopecuroides* has been shown to impede cancer cell growth, induce cell cycle arrest, enhance apoptosis and cellular differentiation, and impede cancer metastasis and invasion. Several mechanisms have been proposed for modulating cancer signaling and molecular pathways or targets based on multitudinous studies in various types of cancerous cells. This review provides an in-depth overview of the antitumor effects of *S. alopecuroides* and the potential targets of 12 alkaloids in *S. alopecuroides* via a pharmacophore mapping approach and offers a scientific basis for the further exploration of the mechanism related to the antitumor effects of this plant.

Keywords: *Sophora alopecuroides*; chemical constituents; alkaloids; pharmacology; antitumor effect

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1. Introduction

Sophora alopecuroides L. is a herbaceous or basal lignified subshrub-like plant of the genus *Sophora*; it is an important desert plant and an important medicinal plant resource(Figure 1). The whole plant, roots, and seeds of *Sophora alopecuroides* (genus: *Sophora* (Leguminosae)) have many important medicinal properties. It is also known as bitter licorice, bitter bean grass, bitter bean root, grass locust (Gansu), Buya (Uyghur language), Hulan-Baoya (Mongolian), etc. It was initially recorded in "Shen Nong's Materia Medica" and "Compendium of Materia Medica" for its role in reducing heat and detoxification, as well as its antibacterial, anti-inflammatory, diuretic, analgesic, and sedative properties. It is now included in the "Chinese Pharmacopoeia", "National Compilation of Chinese Herbal Medicine", "Xinjiang Chinese Herbal Medicine", "Uygur Medicine Journal", etc. [1].

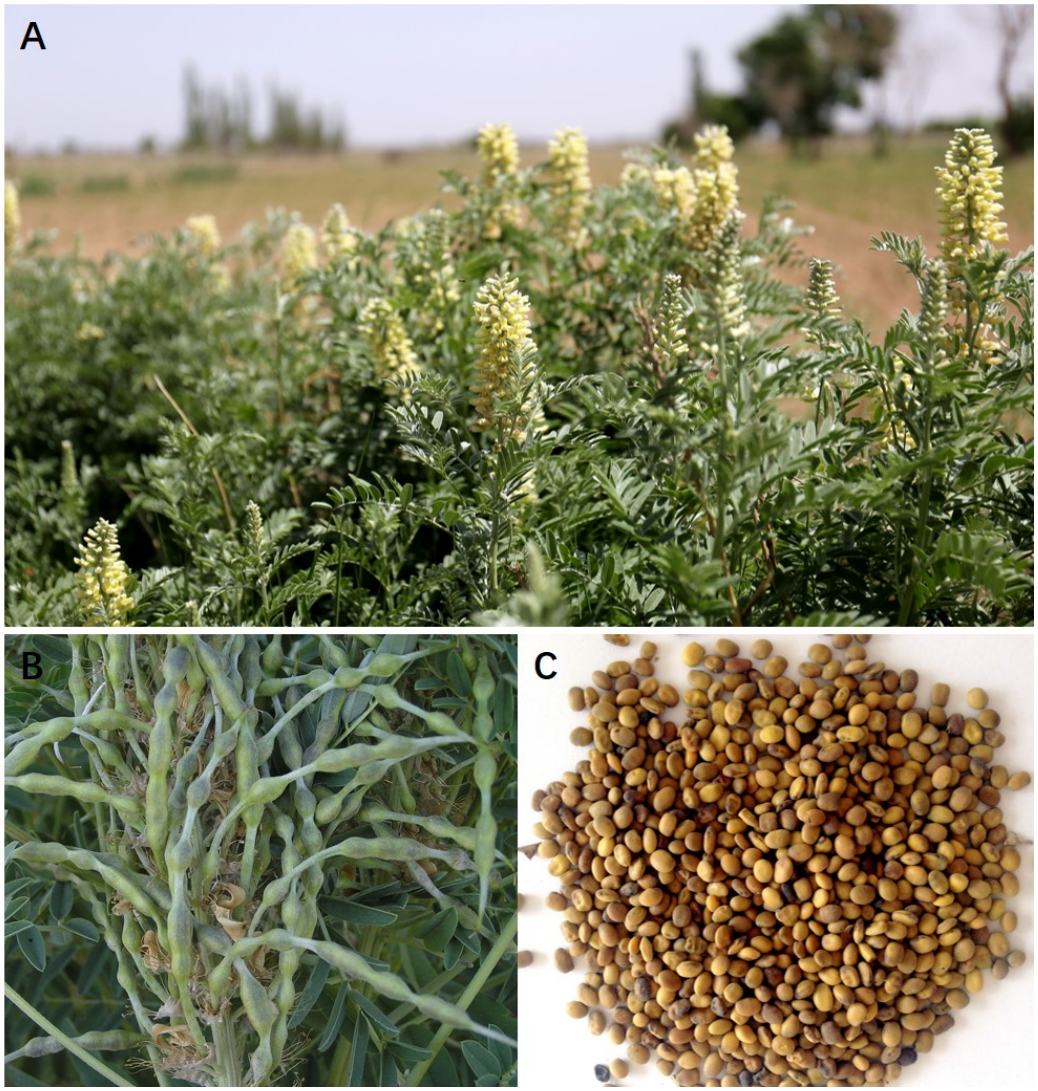

Figure 1. *Sophora alopecuroides* plant characteristics: (**A**) flowering branches and habitats, (**B**) fruits, and (**C**) seeds (bean).

S. alopecuroides (Kudouzi) is widely distributed across west and middle Asia, including Japan, Pakistan, Iran, northern India, and Mongolia. In China, *S. alopecuroides* is mainly distributed in the northwestern part, especially in China's Xinjiang, Gansu, Ningxia, and Inner Mongolia provinces. In China, *S. alopecuroides* is one of the important medicinal materials of sand, and its wild resources are widely distributed. Due to its high medicinal value and ecological function, the rational protection and development and utilization of *S. alopecuroides* resources have attracted more and more attention.

With the continuous research on its medicinal value, many compounds have been extracted and isolated from this plant, and some compounds have been the hot compounds studied in recent years. The extraction of alkaloid components from bitter bean has been reported by many research groups.

Alkaloids are the main chemical compounds and active components of *S. alopecuroides*. Approximately 6.11–8.03% and 8.11% of alkaloids are present in the aerial part and seeds of *S. alopecuroide*. In particular, the remarkable roles of the anticancer activities of alkaloids, such as matrine and oxymatrine, isolated from *S. alopecuroides*, have attracted the attention of many researchers worldwide and become a topic of interest in research [1].

The seeds and grass of *S. alopecuroides* exert a cooling effect and have been used as analgesics, antibacterial, and antirheumatic agents, and for GI disorders in China [2–4]. *S. alopecuroides* is known to possess antitumor [5,6], anti-inflammatory [7], antibacterial [8], antiviral [7], analgesic [9,10], cardioprotective [11], antioxidant [12], antiarrhythmic [13], and neuroprotective [14,15] activities. Numerous studies with different cancerous cell lines have also confirmed the anticancer property and mechanism of *S. alopecuroides*.

Malignant tumors are a serious threat to human health and lead to high morbidity/mortality. Tumor treatment involves molecular targeted therapy and biological therapy using nonspecific cytotoxic drugs and large doses of drugs-induced killer treatment to tumor cells in different phases [16]. The modernization of Traditional Chinese Medicine (TCM) focuses on the study of active components in Chinese medicines or natural products, which display a broad range of clinical effects, including reduced cancer-associated symptoms, increased survival rates, decreased treatment-related toxicity, and prevention of recurrence and metastasis [17].

The information related to this article was collected from the scientific literature databases including PubMed, Google Scholar, Web of Science, Science Direct, Springer, China National Knowledge Infrastructure, published books, PhD and MS dissertations, and other web sources, such as the official website of Flora of China and Yao Zhi website (https://db.yaozh.com/, accessed on 15 March 2022).

This article reviews the antitumor properties of *S. alopecuroides*, its role in cancer prevention, and its possible underlying mechanism. We used PharmMapper, a reverse docking server, to predict the potential targets of 12 alkaloids in *S. alopecuroides*. Pathway analysis was carried out via DAVID and KEGG databases. The Cytoscape3.6.0 software was used to construct the network model of "component-target-pathway" of alkaloids of *S. alopecuroides* to explore the action mechanism of alkaloids of *S. alopecuroides* "multi-component, multi-target-multi-pathway." It provided a scientific basis for the further exploration of the mechanism related to the antitumor effects of *S. alopecuroides*.

2. Phytochemistry

2.1. Alkaloids

Alkaloids are alkaline nitrogen-containing compounds in plants and have obvious physiological activities and are comprise a large number of active ingredients in Chinese herbal medicines. Most of the alkaloids come from the plant kingdom and are widely distributed in higher plants such as papaveraceae, legumes, lilies, and ranunculus [2].

Natural medicines containing alkaloids have complex chemical compositions, including active ingredients and toxic ingredients. In order to improve the therapeutic effect of Chinese herbal medicine, the effective ingredients should be extracted to the maximum extent, and the ineffective and toxic ingredients should be removed. Therefore, how to extract and separate alkaloids from natural products has attracted more and more attention of scholars, thus promoting the continuous improvement and development of their extraction and separation methods [3].

2.2. Extraction and Separation of Alkaloids in S. alopecuroides

Most of the alkaloids exist in the form of salts in Chinese herbal medicine, and a few exist in the form of free bases in plants. There are many methods for extracting and separating alkaloids from Chinese herbal medicines. Choosing a method is mainly based on the nature and existence state of alkaloids in plants, and then the appropriate extraction and separation method is selected.

In general, most alkaloids are extracted by the solvent extraction method, and then the alkaloid compounds are further separated. Therefore, the solubility of alkaloids is an important basis for extraction and purification.

For extraction of alkaloids in *S. alopecuroides*, commonly used alkaloid extraction methods include the acid–water extraction method, alcohol solvent extraction method, lipophilic organic solvent extraction method, percolation method, steam distillation method, microwave extraction method, ultrasonic-assisted extraction method, supercritical fluid extraction method, enzymatic method, etc.

The commonly used separation methods include organic solvent extraction, chromatography, and resin adsorption. With the development and application of new technologies such as rapid preparative chromatography, the process has been greatly simplified, and the efficiency of separation and purification of alkaloids has been improved.

Alkaloids are the major active components present in *S. alopecuroides*, mainly in the aerial part (6.11–8.03%) and seeds (8.11%) (Figure 2). Until now, more than 20 alkaloids have been identified, most of which belong to quinolizidine alkaloids with four heteroatom rings, A, B, C, and D, and possess similar structure. These alkaloids belong to the following four categories: matrine-tetracyclic type, chiconine-tetracyclic type, genistein-tricyclic type, and lupin-bicyclic type alkaloids. Table 1 and Figure 3 show the chemical composition and structure of alkaloids that have been separated and identified. These alkaloids have been extensively studied for their remarkable antitumor activities. Among them, matrine has been used in clinical studies as an antitumor adjuvant to avoid cachexia and enhance the quality of life (QoL) of cancer patients. In fact, these effective components of *S. alopecuroides* may act as potential and promising natural agents to treat cancers.

Table 1. Parts of alkaloids that have been isolated from *S. alopecuroides*.

No.	Compounds	CAS	References	Structure Type
1	Matrine	519-02-8	[1]	Matrine-tetracyclic
2	Sophoridine	6882-68-4	[1]	Matrine-tetracyclic
3	Sophocarpine	6483-15-4	[1]	Matrine-tetracyclic
4	N-oxide-1314-dehydro-sophoridine	77077-09-9	[2]	Matrine-tetracyclic
5	Neosophoramine	52932-74-8	[3]	Matrine-tetracyclic
6	Isosophoramine	6838-34-2	[3]	Matrine-tetracyclic
7	7α-hydroxy-sophoramine	259860-46-3	[4]	Matrine-tetracyclic
8	3α-hydroxy-sophoridine	41645-69-6	[4]	Matrine-tetracyclic
9	Lehmannine	58480-54-9	[4]	Matrine-tetracyclic
10	Sophoramine	6882-66-2	[5]	Matrine-tetracyclic
11	N-hydroxy-sophoridine	32968-81-3	[5]	Matrine-tetracyclic
12	9α-hydroxy-matrine	88509-92-6	[4]	Matrine-tetracyclic
13	14β-hydroxy-matrine	183074-18-2	[2]	Matrine-tetracyclic
14	Aloperine	56293-29-9	[1]	Chrysophylline-tetracyclic
15	N-methyl-aloperine	63128-33-6	[1]	Chrysophylline-tetracyclic
16	N-allyl-aloperine	56595-96-1	[1]	Chrysophylline-tetracyclic
17	Δ11-dehydroaloperine	142808-31-9	[5]	Chrysophylline-tetracyclic
18	Baptifoline	732-50-3	[4]	Chrysophylline-tetracyclic
19	Anagyrine	486-89-5	[3]	Chrysophylline-tetracyclic
20	Cytisine	485-35-8	[1]	Genistein-tricyclic
21	N-methyl-cytisine	82438-76-4	[3]	Genistein-tricyclic
22	N-2-hydroxyethyl-cytisine	41645-70-9	[1]	Genistein-tricyclic
23	Nicotine	54-11-5	[2]	Lupin-bicyclic

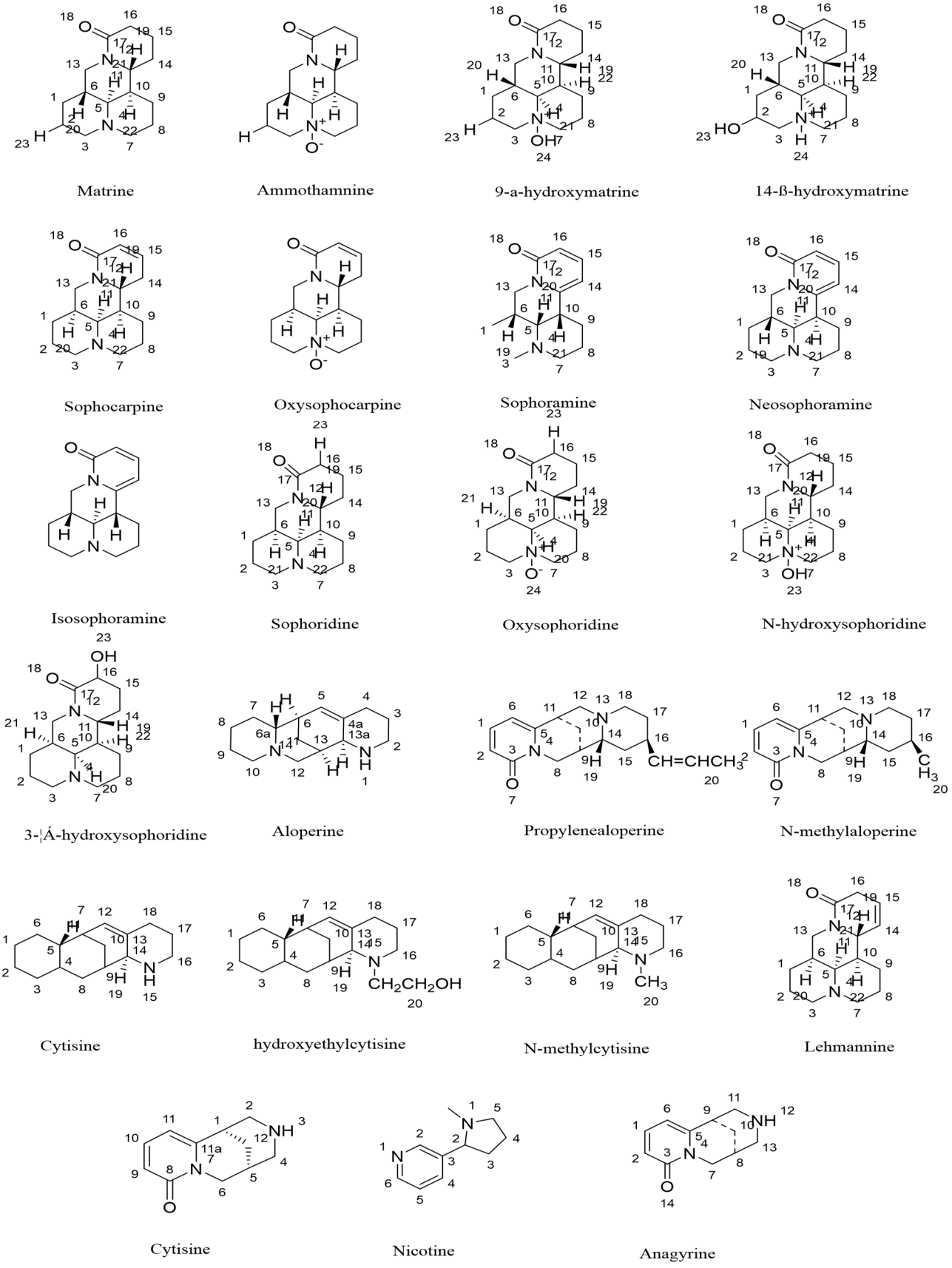

Figure 2. Chemical structure of main alkaloids presents in *S. alopecuroides*.

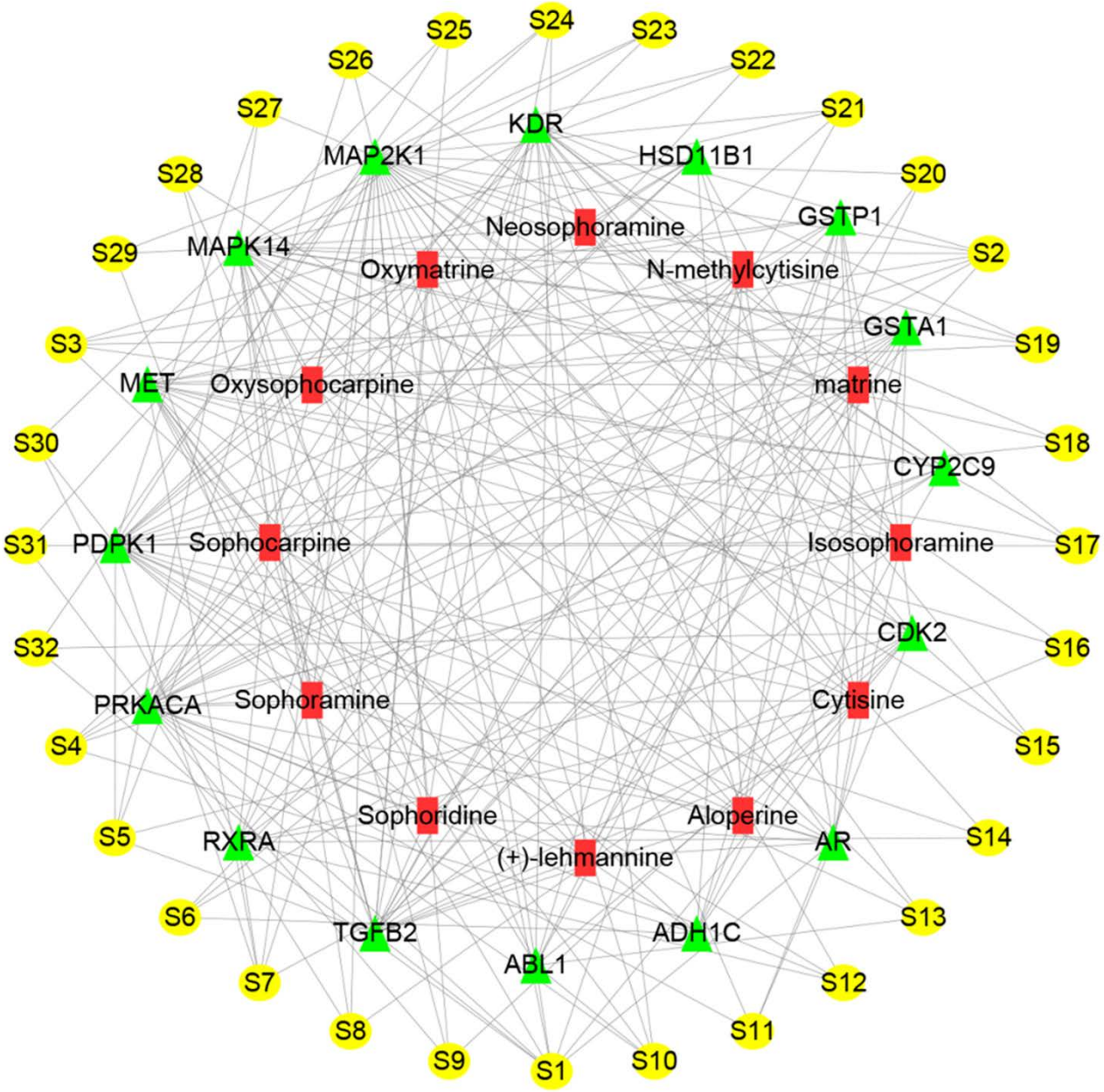

Figure 3. Compound-target-pathway network. The red oblong, green triangles, and yellow circles correspond to SA, target proteins, and pathways, respectively. This network comprises 60 nodes (12 candidate compounds and 16 potential targets) and 262 edges (compound–target interactions).

3. In Vitro Antitumor Activity

3.1. Effect on Tumor Cell Growth

Tumor cells are characterized by uncontrolled growth, and they short-circuit several regulatory pathways to multiply and proliferate [18]. Total alkaloids present in *S. alopecuroides* have been shown to impede growth; treatment with 8.75 mg/mL of total alkaloids of *S. alopecuroides* caused cell death in >50% of HeLa cells, and the cell death rate increased with longer incubation. Additionally, several active ingredients of *S. alopecuroides*, including aloperine [19], cytosine [20,21] matrine [22–25], oxymatrine [26–28], sophoridine [29,30], oxysophoridine [31], and sophocarpine [32], are also known to impede tumor cell growth.

Osteosarcoma is the primary cause of sarcoma-related deaths in children and adolescents. It occurs as a result of irregular differentiation of mesenchymal stem cells [19,33].

Aloperine was found to substantially impede the growth of MG63 and U20S human osteosarcoma cells in a time- and dose-dependent manner by suppressing the PI3K/AKT pathway [19]. Several techniques, such as MTT assay, FACS, WB, and RT-PCR, have been used to study cancer cell inhibition by matrine, including breast cancer (BC) cells (MCF7, MDA-MB-231, BT-474 [34], 4T1 cells [35]), cervical cancer (CC) cells (SiHa, C33A, Caski, HK-2 cells [36]), colorectal cancer (CRC) cells (LoVo [37], HT29 [38], DLD1 cells [39]), lung cancer (LC) cells (A549, LK2 [40], H1299 [41], 95D [42], NCI-H358 cells [43]), prostate cancer (PC) cells (PC-3, RWPE1 [44], LNCaP [45], DU145 cells [46]), urinary bladder cancer (UBC) cells (EJ, T24, BIU, 5637 [47], T24 cells [48]), liver cancer cells (SMMC-7721 [24], HepG2 cells [49]), thyroid cancer (TC) cells (TPC-1 [50]), esophageal cancer cells (Eca-109 [51]), gastric cancer (GC) cells (MKN45 [52]) SGC-7901 [53]), and human pancreatic cancer (PANC) cells (BxPC-3, PANC-1). Oxymatrine was found to substantially impede the growth of A549 LC cells by inducing apoptosis [27]. A study by Wu et al. on oxymatrine-treated MCF-7 and MDA-MB-231 cells impeded growth in a time- and concentration-dependent manner [28]. Meanwhile, oxymatrine-treated MKN-45, BGC823, and SGC7901 GC cell lines showed the suppression of both growth and invasion by inhibiting EGFRp-Tyr845, which reduced the expression of the EGFR/Cyclin D1/CDK4/6, EGFR/Akt, and MEK-1/ERK1/2/MMP2 pathways [54]. Cytisine has been shown to impede the growth of A549, HepG2, Ec109, K562, HL-60, and U937 cells [21]. All these findings indicated that both total alkaloids and monomeric alkaloids of *S. alopecuroides* effectively impeded the growth of various types of cancer cells.

3.2. Antimetastatic and Anti-Invasive Effects

Tumor metastasis and invasion are the primary causes of fatality in cancer patients, and the use of TCM or natural products play a vital role in reducing tumor recurrence and metastasis, prolonging the survival time, and improving the patients' QoL [55]. Matrine, the main component of *S. alopecuroides*, exhibits an antimetastatic effect in metastatic BC, the second leading cause of cancer-related deaths in women worldwide. In a metastatic mice model of 4T1 BC, treatment with matrine (50, 100, and 150 mg/kg) resulted in significant inhibition of tumor metastasis in liver and lung in a concentration-dependent manner [56]. Additionally, aloperine impeded the invasion of human BC cells (MCF-7 and MDA-MB-231) and human osteosarcoma cells (MG-63 and U2OS) based on the results of the transwell invasion assay. They also investigated MMPs, zinc-dependent proteolytic enzymes, which are commonly involved in cell migration and invasion, and found that aloperine not only impeded the transcription of both MMP-2 and MMP-9 genes, but also downregulated their protein expression [19,55]. Oxymatrine effectively inhibited the tumor invasion by downregulating the expressions of phospho-Cofilin (Ser3) and phospho-LIMK1 (Thr508) without altering the total expression of Cofilin and LIMK1 in human GC cells (BGC823 and SGC7901) via the inactivation of AKT/ERK pathway and ROS generation in human GC cells BGC823, and via the inhibition IL-21R-mediated JAK2/STAT3 pathway in GC HGC 27and AGS cells [26,54,57]. Liang et al. showed that oxymatrine impeded the invasion of CRC cells (RKO) by regulating the NF-κB signaling pathway [58]. Additionally, sophorine inhibited the invasion of UMSCC-22B and UM-SCC-47 cells in a dose-dependent manner [32]. These data indicated that alkaloids of *S. alopecuroides* could act as potential therapeutic agents to treat cancers via their antimetastatic and anti-invasive effects.

3.3. Effects on Apoptosis and Autophagy

Apoptosis, a highly selective process of programmed cell death, constitutes the primary mechanism of cell death in cancer treatment [18,19]. Autophagy, i.e., self-feeding, is a degradation process of intracellular catabolism that removes cytoplasmic cellular components surrounded by lysosomes that degrade enzymes to sustain homeostasis and metabolism [18,59]. CC is still the primary cause of cancer-related deaths worldwide, and common treatments include chemotherapy and radiotherapy [36]. Total alkaloids of *S. alopecuroides* were found to substantially increase apoptosis in human CC HeLa cells.

After treatment with total alkaloids of *S. alopecuroides* (6.25 and 12.50 mg/mL), the apoptotic rate was found to be 16.0% and 33.3%, respectively, which were higher than in the control group.

PC is the sixth leading cause of cancer-related mortality globally. Current treatment strategies involve androgen deprivation; however, its therapeutic and preventive effects are limited. Aloperine and oxymatrine are known to promote apoptosis in PC cells (PC3, DU145, and LNCaP) based on the results of annexin V-fluorescein isothiocyanate/propidium iodide staining. With treatment with aloperine (0, 100, and 200 μM) and oxymatrine (0, 4, and 8 μM), a considerable enhancement in the percentage of annexin-V-fluorescein isothiocyanate-positive cells was observed [6,60].

PANC is considered to be one of the most aggressive cancers. There has been a gradual increase in PANC cases, and, consequently, in its death rate. It is estimated that by 2030, PANC will overtake BC, PC, and CRC as the second leading cause of cancer-related death after LC [61]. Treatment of PANC cells (Miapaca-2, PANC-1, and HPDE), with sophoridine (20 μM) for 48 h induced mitochondrial-related apoptosis via the activation of the phosphorylation of ERK and JNK, which killed cancer cells but had low cytotoxicity toward normal cells [30]. Matrine also induced more apoptosis of PANC cells (BxPC-3 and PANC-1) compared with the control, $p < 0.05$, and $p < 0.001$. Additionally, oxymatrine treatment (1 and 2 mg/mL) induced more apoptosis in human PANC cell line PANC-1 than control group ($p < 0.05$) [62,63].

Hepatocellular carcinoma (HCC) is a global health issue. Due to the continuous spread of the hepatitis B and hepatitis C viruses, the overall incidence of liver cancer is still alarmingly high in developing countries and rising steadily in most developed countries. Matrine exhibited significant antitumor activity via inducing apoptosis and autophagy in human HepG2 cells by upregulating Bax expression. Matrine-treated human HepG2 cells had an increased rate of apoptosis (28.91%, 34.36%, and 38.80%) for 0.5, 1.0, and 2.0 mg/mL of matrine, respectively, compared to the control group (0.14%) [25]. Matrine has also been shown to promote apoptosis of liver cancer cells by inhibiting mitophagy and PINK 1/Parkin pathway [49]. Cytisine also induced apoptosis in human HepG2 cells. The results of morphological observation and flow cytometry showed that cytisine induced cytotoxicity via an apoptosis-like mechanism and upregulated the expression of caspase-3 and downregulated the expression of pro-caspase-3. Therefore, cytisine induced apoptosis of HepG2 cells via the mitochondrial pathway [21].

Aloperine is known to modulate apoptosis in human BC cells (MCF-7 and MDA-MB-231) by upregulating the protein expression of Bax, caspase-3, and caspase-9, and downregulating the expression of Bcl-2 [55]. Oxymatrine is known to induce apoptosis in BC cells by modulating apoptosis-related proteins, such as caspase-3, caspase-9, and poly ADP-ribose polymerase [28]. Similar results have also been observed for matrine in human BC cells MCF-7, where treatment with matrine (0.25, 0.5, 1.0, and 2 mg/mL) for 24 h induced apoptosis and the apoptotic rate was approximately 4.17 ± 0.25%, 6.60 ± 0.18%, 15.32 ± 0.21%, 19.63 ± 0.17%, respectively [64]. In other studies, matrine also induced apoptosis via the NF-κB signaling pathway in BC cells MCF7, BT-474, and MDA-MB-231 [65], reducing the protein and mRNA expression of Bcl-2/Bax ratio in the MDA-MB-231 cells [66] and inhibiting the expression of VEGF and downregulating the Wnt/β-catenin signaling pathway [35].

GC is a major public health burden, and it is reported that approximately 1 million GC-related deaths occur annually. Matrine exerts its anti-GC effects by inducing apoptosis in a dose-dependent manner in MKN45 cells; treatment with matrine (0, 0.05, 0.10, 0.25, and 0.50 mg/mL) for 48 h resulted in the apoptotic rates being 0.52%, 1.44%, 1.63%, 6.22% and 26.88%, respectively. The mechanism of action was probably related to an increase in the proapoptotic molecules of the Bcl-2 family [52]. Similar results have also been observed in SGC-7901 cells where apoptosis rates after treatment with matrine (0, 0.5, 1.0 and 2.0 mg/mL) were 0.20 ± 0.13%, 72.92 ± 3.41%, 77.75 ± 2.19%, and 83.28 ± 2.75%, respec-

tively. Moreover, the anticancer effect of matrine was found to be related to autophagy, and its mechanism involved the upregulation of Bax expression [53].

Thus, different alkaloids in *S. alopecuroides* could induce apoptosis and autophagy in the same cancer cells via different mechanisms, demonstrating the inhibitory effect of *S. alopecuroides* on various types of tumors.

3.4. Modulation of Cell Cycle

The cell cycle is a series of events composed of four phases: G1, S, G2, and M phase, which undergo cell division to produce daughter cells. Malignant transformation generally results from disturbance in cell cycle regulation. The cell cycle is regulated by cell cycle-dependent cyclins, CDKs, as well as CDK inhibitors [67,68].

In human osteosarcoma cell line (OS732), treatment with total alkaloids of *S. alopecuroides* (1.5, 3.0, and 4.5 mg/kg) could arrest the cell cycle in the G0/G1 phase, leading to increased accumulation in the G0/G1 phase and a decreased count in the S phase [68]. Aloperine exhibited anticancer effects via the regulation of the cell cycle. Studies by Zhang et al. indicated that aloperine induced G2/M phase arrest in HCT116 human CRC cells. Compared with untreated cells, aloperine-treated cells (0.25 and 0.5 mM) showed an increased number of cells in the G2/M and S phase, while the number of cells in the G0/G1 phase decreased, and the molecular mechanisms were probably associated with a concomitant increase in p21 and p53 expression and a decrease in cyclin D1 and B1 expression [69]. Aloperine also effectively induced cell cycle arrest via the activation of the p53/p21 pathway in PC cells (LNCaP, PC3, and DU145) [6].

Sophocarpine was found to be selectively toxic toward HCC cells. It has been reported that sophocarpine (10 and 1000 μM) could arrest the cell cycle in the G0/G1 phase ($p < 0.05$) while decreasing the cell viability of HCC cells and, consequently, the ratio of cancer stem cells, and EMT induced by TGF-β [70]. Sophoridine also inhibited PANC cells; Zhang et al. analyzed the cell cycle via flow cytometry using Miapaca-2 and PANC-1 cell lines and found that 20 μM sophoridine arrested the cell cycle at S phase via the activation of the JNK and the ERK signaling pathways [30].

Matrine has been shown to exert antitumor effects on several types of cancer cells. Matrine promoted significant G0/G1 accumulation and S and G2/M depletion by upregulating the expression of miR-126 in NSCLC A549 cells. The proportions at the G0/G1 phase were found to be 6.0665 ± 4.3721%, 1.2401 ± 1.4673%, and 1.0201 ± 0.1394% at matrine concentrations of 0, 0.2, and 1.0 mg/mL, respectively [71]. Studies have also shown that the E2F family of transcription factors regulate the G1/S transition in eukaryotic cells [68]. Several studies have found that matrine impedes cell cycle at different phases in various cancer cells, such as GC MKN45 cells [49], MCF-7 cells [72], Eca-109 cells [51], TPC-1 cells [50], MDA-MB-231 cells [66], SGC-7901 cells [53,73,74], HT29 cells, LS 174T, Caco-2, SW1116 and RKO cells [75], LoVo cells [37], HepG2 cells [25,49], EJ and T24 cells [47], PC-3 cells [44,76–78], DU145 cells [76,77], and RWPE1 cells [44].

Additionally, oxymatrine inhibited UBC by inducing cell cycle arrest and apoptosis. Human UBC T24 cells treated with oxymatrine (1.25 and 2.50 mg/mL) for 72 h showed an elevated proportion of cells in the G0/G1 phase from 43.31% to 54.52 and 52.41%, respectively ($p < 0.05$). Its mechanism was probably related to p53-Bax signal transduction and downregulation of survivin expression [79]. These studies indicated that cell cycle-related regulatory factors were involved in the anticancer activity of *S. alopecuroides* and its active components. Several studies have also indicated that oxymatrine inhibited cell cycle progression at different phases in different cancer cells, such as HCC827 cells [48], A549 cells [80], MDA-MB-231 and MCF-7 cells [28], BGC823 cells [26,54], and SW480 cells [81]. Thus, these results suggest that *S. alopecuroides* could regulate the cell cycle to achieve the anticancer effect.

4. In Vivo Effects

When the total alkaloids of *S. alopecuroides* (50, 100, and 200 mg/kg) were applied to the H22 tumor-bearing mice for 12 days, tumor weight in each group was lower than

that in the negative control group, and the inhibition rates were 20.1%, 42.7%, and 52.8%, respectively [82]. In U266 cells in tumor-bearing mice treated with aloperine, the good toleration was observed in addition to suppression of tumor growth and a decrease in IgG2b and bone lesions in a time-dependent manner in 5T33 mice [83]. There have also been reports that at the doses of 30 mg/kg (p.o.), aloperine suppressed the PC3 tumor growth, induced cell cycle arrest, activated the p53/p21 pathway, and induced apoptosis [6].

Matrine was also found to exert an anticancer action in nude mice with minimum side effects via effects on Bcl-2, Bax, and caspase-3. Tumor inhibition rate of the matrine-treated group was 40.36 ± 5.36% and of the oxaliplatin group was 72.58 ± 6.73%, both of which showed significance ($p < 0.01$) compared to the negative control group [75]. Fan et al. used a rat model of colorectal carcinogenesis, which treated with 1, 2-dimethylhydrazine dihydrochloride (30 mg/kg) once per week for 18 weeks, to evaluate the anticancer effects of matrine on CRC. Their results showed that number, weight, and size of CRC rats were effectively inhibited by matrine via inhibition of HMGB1 signaling to decreased IL-6, TNF-α, p53, and HMGB1 [84].

In 4T1 tumor-bearing mice treated with matrine (50 mg/kg and 100 mg/kg, i.p), an induction of apoptosis was observed in addition to a suppression of tumor growth and a reduced expression of Wnt1, β-catenin, cyclin D1, and c-Myc substantially [35]. BxPC-3 tumors were established in mice after treatment with matrine (50, 100, and 200 mg/kg, i.p) for 18 days, and the tumors in the matrine-treated group (100 and 200 mg/kg) were smaller than in the control group ($p < 0.01$), while bodyweight and histological analysis of livers and kidneys did not substantially differ from the control mice. Furthermore, its mechanism may be related to the downregulation of the expression of PCNA, and it induced cell apoptosis by reducing the ratio of Bcl-2/Bax and upregulating Fas caspases-8, -3, and -9 [63].

In an LC xenograft model, tumor size and tumor weight were remarkably decreased by oxymatrine, along with the suppression of constitutive activation of STAT5 via nuclear localization, the activation of JAK1/2 and c-Src, as well as STAT5 binding to DNA in A549 cells and abrogated IL-6-induced STAT5 phosphorylation in H1299 cells [80]. Ye et al. investigated the in vivo effects of oxymatrine and cisplatin on NSCLC; after cotreatment of oxymatrine with cisplatin, the weight and volume were substantially decreased, and the inhibition rate was as high as 94.19%. Moreover, it synergistically increased the CD8+/Treg ratio [85]. Oxymatrine and oxaliplatin can also synergistically impede the colon carcinoma through the PI3K/AKT/mTOR pathway [81]. The effects of sophoridine on various types of cancer were significant. For pancreatic tumor-bearing BALB/c homozygous (nu/nu) nude mice treated with 20 or 40 mg/kg (i.p) sophoridine for 21 days, tumor volume and weight were markedly reduced. Furthermore, ERK and JNK were activated, proliferative cells were decreased, and apoptotic cells were increased in xenograft tumor tissues [30]. In SW480 tumor-bearing nude mice, the sophoridine-treated group also suppressed the weight and volume, and there was absence of substantial difference in inhibition rate between the sophoridine-treated group and the positive control group (5-Fu) ($p > 0.05$) [29]. The results in vivo indicated that alkaloids of *S. alopecuroides* could effectively impede the growth of all kinds of tumors with minimum side effects, and its inhibitory effect was similar to that of 5-Fu and oxaliplatin; additionally, oxymatrine and cisplatin synergistically enhance antitumor efficacy, so the antitumor effect of *S. alopecuroides* was quite significant. All these findings indicated that *S. alopecuroides* and its alkaloids may be the novel effective candidate for the treatment of cancer.

5. Antitumor Mechanism

5.1. Research on the Cellular Mechanism of Antitumor Effect

5.1.1. Inhibiting Tumors by Affecting the Cell Cycle

Matrine is known to induce apoptosis in human ovarian cancer cells (HO8910) by inducing S + G2/M-phase arrest [86]. A study treated HCC cells (SMMC-7721) with different concentrations of oxymatrine, followed by MTT assay and FACS to study cell growth and DNA distribution, respectively. At oxymatrine > 2.5 mg/mL, significant

cytotoxicity was observed, indicating that oxymatrine increased the percentage of cells in the G0/G1 phase and prevented cells from entering the S phase [87]. OMT blocked the SMMC-7721 cell cycle in the G2/M and S phases, preventing their entry into the G0/G1 phase, thereby inducing the apoptosis of SMMC-7721 cells [88]. After 72 h of treatment with matrine, 64.6% of tumor cells were blocked in the G1 phase, thus reducing the cell count in the S phase, indicating that matrine could impede cell growth. Moreover, the regulation of cell growth mainly occurs through the inhibition of CDK activity in the G1 and G2 phases, which stops the cell cycle by inhibiting the division of DNA or that of copied DNA into daughter cells, thereby inhibiting cell growth [89].

5.1.2. Alter Cell Signal to Impede Tumor

Studies have shown that MA partially impedes the activity of Ca^{2+}/Mg^{2+}-ATPase, while OM exerts no obvious effect, which might be related to the fact that although both drugs act on the membrane structure, MA destroys function and OM stabilizes the membrane structure [90]. After treatment with matrine, there was a rapid increase in Ca^{2+} concentration in K562 cells both with/without calcium. It shows that matrine triggered immediate changes and long-term effects in intracellular Ca^{2+} concentration in K562 cells, suggesting that calcium signal acted as an early signal for matrine to induce differentiation and apoptosis in K562 cells [91]. Treatment of K562 cells with matrine (0.1 mg/mL) increased the activity and expression of phospholipase A2. Phospholipase A2, as an important cell signaling molecule, works with matrine to promote K562 cell differentiation and signal transduction by regulating its activity and expression. Reversible phosphorylation of proteins is a major regulatory factor in important cell signaling processes that are associated with cell behaviors, such as cell growth and differentiation [92]. During matrine-induced differentiation of K562 cells, a change in at least six tyrosine protein phosphorylation levels was observed, of which four were upregulated and two were downregulated; an increase in the concentration of matrine induced the upregulation of four tyrosine protein phosphates. The decreased levels of chemokine indicated that intracellular tyrosine protein phosphorylation played an important role in matrine-induced K562 cell differentiation in cell signaling pathways [93].

5.1.3. Altering the Cytoskeleton to Impede Tumors

Cell scaffolds not only participate in the maintenance of cell morphology, but also participate in cellular signaling and transport of substances and connect with the outer cell membrane. Structure and cell matrix connections influence cell adhesion [94]. Studies have shown that matrine induces cytoskeletal aggregation during the differentiation of liver cancer cells. Reduced contact with the cell membrane is known to reduce tumor cell adhesion, reduce signal transmission between tumor cells, and help tumor cells differentiate into normal cells. During the differentiation of hepatoma cells into normal hepatocytes, the cytoskeleton changes from multiple regular filaments into disordered deep-stained bunches separated from the membrane. Matrine promotes the disaggregation of cellular microfilaments and microtubules, thereby shortening and thickening, to adapt to changes in cell morphology [95]. Depending on the action of the drug, the composition of the cytoskeleton changes, the skeletal proteins that help differentiation increase, and the skeletal proteins that do not help differentiation decrease. Therefore, when the induced drug is removed, the cytoskeleton gradually becomes normal [96]. At certain concentrations of matrine, SMMC-7721 cells showed growth inhibition, changes in regular morphology, small nucleus, substantially reduced nucleolus, and vacuolar cytoplasm, similar to normal liver cells [97].

5.2. Research on the Molecular Mechanism of Antitumor Effect

5.2.1. Regulate the Expression of Apoptosis-Related Genes

Immunohistochemistry and image analysis techniques showed that matrine could substantially reduce the expression of Bcl-2 protein produced by gastric adenocarcinoma cells (SGC-7901), thereby weakening the antiapoptotic ability of these cells. It was spec-

ulated that matrine inhibited the growth of GC cells. The suppressed expression of Bcl-2 proto-oncogene was related to apoptosis [98]. The use of matrine to induce the differentiation and apoptosis of human hepatoma cells indicated that the mechanism was probably related to the upregulated expression of HepG2 cell cycle negative regulators P21, P16, P27, and Rb and the downregulated expression of positive regulator Cy-clinD1 [99]. Matrine not only inhibited the growth of tumor cells and promoted their benign differentiation, but also induced tumor cell apoptosis. Its mechanism of action was mainly related to an upregulated/downregulated expression of various subunits in cyclin, CDK, and CDK inhibitors (CDKi) [99,100], which suppressed c-myc, BCL-2, and other oncogenes, and upregulated the activity of N-ras, p53, and other tumor suppressor genes [101–103], which enhanced mRNA and protein expression of Fas, and mediated apoptosis via the Fas/FasL-FADD pathway [104,105]. Sophoridine induced apoptosis in HCC cells, and its molecular mechanism was related to the downregulated expression of Bcl-2 and survivin genes and upregulated expression of caspase-3 gene in HepG2 cells [106].

5.2.2. Inhibition of Tumor Drug Resistance by Regulating Drug-Resistant Genes

The effect of matrine and vincristine on multi-drug-resistant cells K562/Vin reversed the resistance of K562/Vin cells toward vincristine and reduced the expression of drug resistance factor, P-glycoprotein (P-pg), on the cell surface to promote cell differentiation and induce apoptosis [107]. The construction of recombinant adenovirus microspheres containing the antisense multidrug resistance-associated protein (MRP) gene could effectively reverse multidrug resistance in HCC cells in vitro. In vivo experiments showed that after injecting PELA microspheres into the hepatic artery, there was a significant decrease in the size of the tumor. Thus, gene therapy for extended durations could help impede tumor tissue growth in large quantities [108]. Additionally, antisense phosphorothioate oligonucleotides were shown to partially reverse the drug resistance in liver cancer cells in nude mice [109].

5.3. Impede Tumor Invasion and Metastasis

The cell adhesion, movement, degradation, and invasion tests confirmed matrine-induced inhibition of the metastatic invasion of SMMC-7721 cells. Thus, treatment with 0.8 g/L matrine resulted in significant reduction in the invasion of transmembrane cells compared to that of the control group, confirming that matrine inhibited cell invasion and metastasis through multiple mechanisms [110]. The mechanisms involved in inhibiting tumor metastasis were probably related to changes in HPSE-mRNA expression, infiltrating genes, and epidermal growth factor [111]. Matrine substantially impeded the adhesion and invasion ability of A375 cells by downregulating the expression of HPSE-mRNA in malignant melanoma A375 cells [112]. Gefitinib, an EGFR tyrosine kinase inhibitor, substantially inhibited the growth of mouse liver cancer cells (H22), and the inhibitory effect was strengthened when used along with cisplatin [113].

5.4. Inhibition of Telomerase Activity

Matrine induced a substantially downregulated expression of the cell telomerase catalytic element, hTERT-mRNA, along with a decrease in telomerase activity in liver cancer and intestinal cancer cells [114,115]. Matrine, at $\geq$500 μg/mL, was found to impede the growth of HepG2 cells; at 750 μg/mL, it was found to impede telomerase activity and downregulate the expression of hTERT promoter. One of the mechanisms by which matrine impeded the growth of HepG2 cells involved the regulation of the expression of hTERT and its effect on the telomerase activity [116].

5.5. Enhanced Immunity

The use of oxymatrine on a Hep-A-22 liver cancer mouse model showed that it possessed a tumor suppressing effect and could stimulate IL-2 secretion, increasing the NK cell activity and activating immune cells. Since tumor-bearing mice usually had a reduced immune function, oxymatrine exhibited an immune enhancement effect, showing that the

antitumor effect of oxymatrine was related to the regulation of immune function [117]. Compound Kushen Injection is known to be a good immunomodulator [118]. Matrine injection was used to treat primary liver cancer. Post-treatment, peripheral blood levels of CD^{3+}, CD^{4+}, CD^{8+}, CD^{4+}/CD^{8+}, and NK cells were substantially improved compared to the control group. Compound Kushen Injection could substantially restore the patients' immune function after interventional treatment [119].

5.6. Delaying Induced Canceration and Preventing Chronic Inflammation from Developing into Cancer

Matrine was administered to rats with diethylnitrosamine (DEN)-induced hepatic cancer. The number and size of liver cancer nodules in the experimental group were substantially lower compared with the control group, suggesting that matrine could not completely block the DEN induction. However, long-term oral administration of small doses could delay the process of DEN-induced liver cancer in rats [120]. Sodium dextran sulfate (DSS) was used to induce colitis in rats, and then the diseased rats were fed with oxymatrine to compare the disease activity (DAI) and pathology scores of the treatment group and the control group. There was a significant improvement in the colitis and pathological changes in the treatment group compared to the control group, suggesting that oxymatrine reduced the induced colitis symptoms and intestinal mucosal damage, which implied that the matrine alkaloids probably exhibited partial anti-inflammatory activity to prevent the occurrence of related tumors.

6. Products in Clinical Trials

Matrine injection as an adjuvant antitumor drug has been clinically used to reduce bone marrow suppression of chemotherapy, relieve gastrointestinal reaction, strengthen the effect of chemotherapeutic drugs, reduce the toxic side effects of drugs, reduce tumor transplantation, reduce the risk of recurrence, etc. [121]. Du et al. studied the impact of matrine injection on chemosensitization, QoL, and immune function in patients with cervical cancer. Seventy patients with CC were randomly divided into two groups (n = 35 each). Patients in the control and observation groups received cisplatin plus docetaxel chemotherapy and cisplatin combined with docetaxel chemotherapy and matrine injection, respectively. After 12 weeks of treatment, the observation group showed a treatment efficacy rate of 94.29%, which was substantially higher compared with the control group's rate of 71.43%. Additionally, the serum levels of CA125 and IL-6 were substantially lower than those before intervention, and IL-18 and TNF-γ were substantially higher than those before intervention. Before intervention, the levels of CD^{4+} and CD^{8+} were decreased in the observation group; at the same time, QoL, KPS, and score of evaluation of QoL increased [122].

Lu et al. also studied the effects of matrine injection on serum tumor markers in 100 patients with CC after neoadjuvant chemotherapy. The patients were randomly divided into a matrine-treated group (n = 50) and a control group (n = 50). The remission rate of the matrine-treated group (88.00%) was substantially elevated compared to the control group's (72.00%) ($p < 0.05$), and the levels of CA125, CEA, CA199, and SCC-A in both groups were substantially downregulated compared to those before treatment ($p < 0.05$). After treatment, the levels of CA125, CEA, CA199, and SCC-A in the matrine-treated group were 36.71 ± 8.36, 30.74 ± 3.29, 30.73 ± 8.23, and 0.93 ± 0.12 IU/mL, respectively, and these levels were substantially downregulated compared to those in the control group, at 41.09 ± 9.36, 35.62 ± 3.35, 36.08 ± 9.32, and 1.09 ± 0.10 IU/mL. Additionally, the incidence of leukopenia, nausea, vomiting, renal injury, and alopecia in the matrine-treated group was 10.00%, 18.00%, 12.00%, and 6.00%, respectively, which were substantially lower than those in the control group, at 26.00%, 36.00%, 28.00%, and 20.00% ($p < 0.05$) [123].

7. Network Pharmacology

7.1. Network Pharmacology Construction and Analysis of Alkaloids in S. alopecuroides

The alkaloid components in *S. alopecuroides* were searched and collected from the related literature and the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed/,

accessed on 1 October 2021). The ingredients for *S. alopecuroides* were retrieved using the following inquiry terms: *S. alopecuroides.* L, Kudouzi.

It was reported that some components with good pharmacokinetic properties were based on the ADME system, including predict oral bioavailability (PreOB) and Caco-2 permeability (PreCaco-2). OB describes the efficiency of drug delivery to the systemic circulation and was estimated using OBioavail1.1 in the TCMSP database [124,125]. Additionally, for oral drugs, one of the best issues is crossing the intestinal epithelial barrier, which determines the rate and extent of absorption and eventually affects its bioavailability [126]. Thus, the Caco-2 permeability prediction model, preCaco2, was used in the TCMSP database [86]. Finally, compounds with OB > 30% and Caco2 > 0.4 cm/s were viewed as active components for further analysis. The OB values of aloperine, isosophoramine, oxysophocarpine, and neosophoramine, were unavailable, but all of them were generally expected to be the active components in *S. alopecuroides*. These compounds were also viewed as candidate compounds [127].

The chemical compounds and protein interactions were derived from PharmMapper, a reverse docking server to build the compound-target collaboration profiles in *S. alopecuroides* [128]. These sdf documents of ingredients were downloaded from the PubChem database and transferred to the PharmMapper with all parameters set to default values. The biological targets related to tumor were selected from the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/, accessed on 20 February 2021) [129,130].

Potential targets and the Uniprot ID were, respectively, imported into the DAVID (https://david.ncifcrf.gov/, accessed on 20 February 2021) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (http://www.genome.jp/kegg/, accessed on 20 February 2021) following instructions to predict the related pathways. The compound-target-pathway networks of *S. alopecuroides* were constructed by Cytoscape 3.6.0 software (Bethesda, MD, USA; http://www.cytoscape.org/, accessed on 20 February 2021) to understand the complex relationships involved in diseases. In this network, nodes represented compounds, targets, and related-tumor pathway, and edges represented the compound–target interactions.

7.2. ADME Screening of Alkaloids in S. alopecuroides

The eight ingredients in *S. alopecuroides* with favorable pharmacokinetic properties were predicted using an in silico-based method in the TCMSP database (Table 2). Additionally, another four compounds below this specification were also considered to be active compounds in *S. alopecuroides* [127]. Thus, twelve compounds were selected for further analysis.

Table 2. Pharmacological and molecular properties of 8 alkaloids in *S. alopecuroides*.

Compound ID	Molecular Weight	Compound Name	Oral Bioavailability (%)	Predicted Caco-2 Permeability
MOL003680	248.41	Sophoridine	60.07	1.13
MOL003676	244.37	Sophoramine	42.16	1.43
MOL003627	246.39	Sophocarpine	64.26	0.99
MOL003680	264.41	Oxymatrine	60.07	1.13
MOL003632	204.30	N-methylcytisine	76.70	1.06
MOL005944	248.41	Matrine	63.77	1.39
MOL005945	190.27	Cytisine	69.40	1.01
MOL006566	246.39	(+)-Lehmannine	58.34	1.21

7.3. Identification of Potential Related-Tumor Targets

The compounds and protein interaction investigation results demonstrated that 49 intracellular targets were predicted to interact with the 12 components of *S. alopecuroides* (Table 3). Among them, 16 were viewed as potential tumor-related targets (Table 4). It also predicted that 32 signaling pathways were related to these 49 targets, in which 5 pathways

were related to tumors, including pathways in cancer, proteoglycans in cancer, chemical carcinogenesis, PC, and NSCLC.

Table 3. All targets of 12 alkaloids in *S. alopecuroides*.

Rank	Uniprot ID	Target Genes	Target Protein
1	Q16539	MAPK14	Mitogen-activated protein kinase 14
2	P61812	TGFB2	Transforming growth factor beta-2
3	P50579	METAP2	Methionine aminopeptidase 2
4	P14061	HSD17B1	Estradiol 17-beta-dehydrogenase 1
5	Q14994	NR1I3	Nuclear receptor subfamily 1 group I member 3
6	P19793	RXRA	Retinoic acid receptor RXR-alpha
7	Q02750	MAP2K1	Dual specificity mitogen-activated protein kinase 1
8	P29373	CRABP2	Cellular retinoic acid-binding protein 2
9	P00918	CA2	Carbonic anhydrase 2
10	P27487	DPP4	Dipeptidyl peptidase 4
11	P55263	ADK	Adenosine kinase
12	P07900	HSD90AA1	Heat shock protein HSP 90-alpha
13	P18031	PTPN1	Tyrosine-protein phosphatase nonreceptor type 1
14	P08263	GSTA1	Glutathione S-transferase A1
15	P05413	FABP3	Fatty acid-binding protein, heart
16	O15540	FABP7	Fatty acid-binding protein, brain
17	Q02127	DHODH	Dihydroorotate dehydrogenase (quinone), mitochondrial
18	P24941	CDK2	Cyclin-dependent kinase 2
19	P15121	AKR1B1	Aldose reductase
20	P00519	ABL1	Tyrosine-protein kinase ABL1
21	P28845	HSD11B1	Corticosteroid 11-beta-dehydrogenase isozyme 1
22	P49354	FNTA	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha
23	P35968	KDR	Vascular endothelial growth factor receptor 2
24	P10275	AR	Androgen receptor
25	Q96RI1	NR1H4	Bile acid receptor
26	P09960	LTA4H	Leukotriene A-4 hydrolase
27	Q08499	PDE4D	cAMP-specific 3′,5′-cyclic phosphodiesterase 4D
28	P10828	THRB	Thyroid hormone receptor beta
29	P62942	FKBP1A	Peptidyl-prolyl cis-trans isomerase FKBP1A
30	P00734	F2	Prothrombin
31	P16442	ABO	Histo-blood group ABO system transferase
32	P00326	ADH1C	Alcohol dehydrogenase 1C
33	P00517	PRKACA	cAMP-dependent protein kinase catalytic subunit alpha
34	P14324	FDPS	Farnesyl pyrophosphate synthase
35	P00390	GSR	Glutathione reductase, mitochondrial
36	O15530	PDPK1	3-phosphoinositide-dependent protein kinase 1
37	P20248	CCNA2	Cyclin-A2
38	P56817	BACE1	Beta-secretase 1
39	P09211	GSTP1	Glutathione S-transferase P
40	P45452	MMP13	Collagenase 3
41	P49638	TTPA	Alpha-tocopherol transfer protein
42	P11712	CYP2C9	Cytochrome P450 2C9
43	P27707	DCK	Deoxycytidine kinase
44	P04150	NR3C1	Glucocorticoid receptor
45	P08581	MET	Hepatocyte growth factor receptor
46	O75469	NR1I2	Nuclear receptor subfamily 1 group I member 2
47	P13631	RARG	Retinoic acid receptor gamma
48	Q9BY41	HDAC8	Histone deacetylase 8
49	P49888	SULT1E1	Estrogen sulfotransferase

Table 4. The information of 16 potential related-tumor targets in *S. alopecuroides*.

Rank	Uniprot ID	Target Gene	Target Protein
1	P19793	RXRA	Retinoic acid receptor RXR-alpha
2	P10275	AR	Androgen receptor
3	P35968	KDR	Vascular endothelial growth factor receptor 2
4	P08263	GSTA1	Glutathione S-transferase A1
5	O15530	PDPK1	3-phosphoinositide-dependent protein kinase 1
6	P08581	MET	Hepatocyte growth factor receptor
7	Q02750	MAP2K1	Dual specificity mitogen-activated protein kinase 1
8	P09211	GSTP1	Glutathione S-transferase P
9	P24941	CDK2	Cyclin-dependent kinase 2
10	Q16539	MAPK14	Mitogen-activated protein kinase 14
11	P61812	TGFB2	Transforming growth factor beta-2
12	P00519	ABL1	Tyrosine-protein kinase ABL1
13	P28845	HSD11B1	Corticosteroid 11-beta-dehydrogenase isozyme 1
14	P00326	ADH1C	Alcohol dehydrogenase 1C
15	P00517	PRKACA	cAMP-dependent protein kinase catalytic subunit alpha
16	P11712	CYP2C9	Cytochrome P450 2C9

7.4. Pathway Analysis and Network Building

The DAVID (https://david.ncifcrf.gov/, accessed on 20 February 2021) and KEGG databases (http://www.genome.jp/kegg/, accessed on 20 February 2021) were used to further explore the 16 identified targets, following the online instructions to predict the related pathways (Table 5). Sixteen targets participated in 32 KEGG pathways, including pathways in cancer, metabolism of xenobiotics by cytochrome P450, proteoglycans in cancer, chemical carcinogenesis, PC, and PI3K-Akt signaling pathway. Based on target identification and pathway investigation, an entire network was built utilizing Cytoscape 3.6.0. The interaction network had 60 nodes and 262 edges (Figure 2). The red oblong, green triangles, and yellow circles were related to *S. alopecuroides*, target proteins, and pathways, respectively.

Table 5. Information of related pathways from 16 potential related-tumor targets in *S. alopecuroides*.

Rank	Pathway Name	Pathway ID	Count	Pathway Classifications
S1	Pathways in cancer	hsa05200	9	Human Diseases; Cancers
S2	Proteoglycans in cancer	hsa05205	7	Human Diseases; Cancers
S3	Metabolism of xenobiotics by cytochrome P450	hsa00980	5	Metabolism; Xenobiotics Biodegradation and Metabolism
S4	Chemical carcinogenesis	hsa05204	5	Human Diseases; Cancers
S5	FoxO signaling pathway	hsa04068	5	Environmental Information Processing; Signal Transduction
S6	Drug metabolism-cytochrome P450	hsa00982	4	Metabolism; Xenobiotics Biodegradation and Metabolism
S7	PI3K-Akt signaling pathway	hsa04151	6	Environmental Information Processing; Signal Transduction
S8	Progesterone-mediated oocyte maturation	hsa04914	4	Organismal Systems; Endocrine System
S9	Prostate cancer	hsa05215	4	Human Diseases; Cancers
S10	Ras signaling pathway	hsa04014	5	Environmental Information Processing; Signal Transduction
S11	Oocyte meiosis 4	hsa04114	4	Cellular Processes; Cell Growth and Death
S12	Thyroid hormone signaling pathway	hsa04919	4	Organismal Systems; Endocrine System
S13	Neurotrophin signaling pathway	hsa04722	4	Organismal Systems; Nervous System
S14	Nonsmall cell lung cancer	hsa05223	3	Human Diseases; Cancers
S15	VEGF signaling pathway	hsa04370	3	Environmental Information Processing; Signal Transduction
S16	Renal cell carcinoma	hsa05211	3	Human Diseases; Cancers
S17	Focal adhesion 4	hsa04510	4	Cellular Processes; Cellular Community-eukaryotes

Table 5. *Cont.*

Rank	Pathway Name	Pathway ID	Count	Pathway Classifications
S18	Fc epsilon RI signaling pathway	hsa04664	3	Organismal Systems; Immune System
S19	Rap1 signaling pathway	hsa04015	4	Environmental Information Processing; Signal Transduction
S20	Chronic myeloid leukemia	hsa05220	3	Human Diseases; Cancers
S21	MAPK signaling pathway	hsa04010	4	Environmental Information Processing; Signal Transduction
S22	GnRH signaling pathway	hsa04912	3	Organismal Systems; Endocrine System
S23	T cell receptor signaling pathway	hsa04660	3	Organismal Systems; Immune System
S24	MicroRNAs in cancer	hsa05206	4	Human Diseases; Cancers
S25	Toxoplasmosis	hsa05145	3	Human Diseases; Infectious Diseases: Parasitic
S26	Serotonergic synapse	hsa04726	3	Organismal Systems; Nervous System
S27	Sphingolipid signaling pathway	hsa04071	3	Environmental Information Processing; Signal Transduction
S28	Cell cycle	hsa04110	3	Cellular Processes; Cell Growth and Death
S29	Osteoclast differentiation	hsa04380	3	Organismal Systems; Development
S30	Hepatitis C	hsa05160	3	Human Diseases; Infectious Diseases: Viral
S31	Insulin signaling pathway	hsa04910	3	Organismal Systems; Endocrine System
S32	Hepatitis B	hsa05161	3	Human Diseases; Infectious Diseases: Viral

8. Toxicity of *S. alopecuroides*

In natural medicines, especially those containing alkaloids, the active ingredients also exhibit certain toxicity while exerting therapeutic effects. Therefore, the active ingredients are also 'toxic ingredients' to a certain extent. For *S. alopecuroides*, people can be poisoned by its seeds when signs of poisoning, such as dizziness, headache, nausea, vomiting, palpitations, fidgeting, abdominal distension, and pale complexion, appear.

At present, the toxicity study of *S. alopecuroides* and its active components is still in the exploratory stage and focused on acute toxicity study. Therefore, in addition to the classical toxicological evaluation, a study on chronic toxicity, mechanism, and toxicokinetics should be strengthened in the future and provide scientific explanation for its toxicity and safety application.

9. Future Perspectives and Conclusions

In recent years, with the continuous exploration of natural drugs, it has become one of the hot spots of antitumor drug research to find natural antitumor drugs with significant antitumor activity and low toxicity. Literature search shows that the current research on the antitumor activity of *S. alopecuroides* mainly focuses on the study of extracts and some common compounds and their related mechanisms. In this paper, through a systematic literature review, based on the alkaloids of *Sophora alopecuroides* and its extraction and separation, studies were reviewed, and the antitumor active substances, pharmacological effects, molecular mechanisms, and other aspects of a more systematic review were reviewed for the research and comprehensive utilization of this plant to provide a new reference.

The above discussions on the antitumor activity of *S. alopecuroides* or its alkaloids are mostly based on previous nonclinical and clinical studies which have identified several vital targets involved in the anticancer mechanism, such as inhibition of tumor cell growth, invasion, metastasis, induction of cell apoptosis, autophagy, and modulation of cell cycle. Due to the extensive antitumor activities of alkaloids in *S. alopecuroides* in different cells or animal models, there is a chance that multiple mechanisms might be involved even in the same experimental system, and an alkaloid may exhibit cancer inhibitory activities via multiple mechanisms.

The significant antitumor effects of alkaloids in *S. alopecuroides* against various kinds of cancer cells together with the conspicuous efficacy observed in clinical medication provide a basis for the development of alkaloids in *S. alopecuroides* as the new effective anticancer

drug candidate. Despite substantial progress in the development of antitumor activities and the molecular mechanism of alkaloids in *S. alopecuroides*, the risk of developing resistance by these agents remains a serious challenge. The active components in *S. alopecuroides* are alkaloids, which have emerged as promising anticancer agents. While diverse antitumor activities and anticarcinomatous mechanisms of these alkaloids in *S. alopecuroides* have been explored, more studies are required to further explore its anticancer activity.

Thus, the antitumor activity of *S. alopecuroides* and its alkaloids in animal or cell models, as well as underlying mechanisms, have been extensively studied. Although *S. alopecuroides* and its alkaloids exhibit significant antitumor activity, only a few of them have been studied clinically. Therefore, future research should mainly focus on clinical research of these active components. Furthermore, the appropriate dosage form and exact mechanisms still need to be estimated to provide more accurate evidence for clinical applications. Although there are many alkaloids of *S. alopecuroides*, which can be used as clinical drug candidates, their identification still remains a challenge.

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Abbreviations

5-Fu, 5-fluorouracil; HMGB1, High-Mobility Group Box Protein 1; IL-6, Interleukin 6; TNF-α, Tumour Necrosis Factor alpha.; DAVID, Database or Annotation, Visualization and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes; PI3K-AKT, Phosphatidylinositol-3kinase/protein kinase B; RT-PCR, Real-time Polymerase Chain Reaction; EGFR, Epidermal growth factor receptor; CDK, Cyclin-Dependent Kinases; MEK, mitogen-activated protein kinase; JAK, Janus Kinase; ERK, extracellular signal regulated kinase; MMP, mitochondrial membrane potential; IL-21R, Interleukin 21 receptor; STAT, signal transducer and activator of transcription; ROS, Reactive oxygen species; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2 Associated X Protein; PINK 1, PTEN-Induced putative kinase; NF-κB, nuclear factor kappa B; G1, Gap 1; S, Synthetic; G2, Gap 2; M, Mitotic; TGF-β, transforming growth factor-β; PCNA, proliferating cell nuclear antigen.

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