Pharmacological Potential of Spatholobus suberectus Dunn on Cancer

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Spatholobus suberectus Dunn (SSD, Leguminosae) is a perennial woody vine, indigenous to tropical and subtropical forests in China and other Southeast Asian countries. The vine stem of SSD is called “Jixueteng” (literally means ‘chicken blood vines’) in Chinese, due to the blood-like outflow from its vine stem when it is injured. SSD has been extensively employed in Traditional Chinese Medicine to treat several ailments. SSD and its active compounds are effective therapeutic agents for treating a variety of diseases with negligible side effects. SSD has been frequently attributed to having antioxidant, anti-diabetic, anti-inflammatory, hematopoietic, neuroprotective, antimicrobial, and anticancer properties.

Keywords: Spatholobus suberectus Dunn ; phytochemistry ; pharmacological activity ; cancer prevention

1. Introduction

The vine stem of Spatholobus suberectus Dunn (SSD, Leguminosae) has been widely employed in Chinese Medicine to treat hematopoiesis, anemia, rheumatism, and menoxenia in Chinese society for nearly years [1]. (**Figure 1**). It is most commonly used as a food additive in southern Asian regions for wine, tea, and soup. In clinical practice, herbal medicine is highly effective in terms of its bioavailability and dose effectiveness, as it contains active ingredients with reliable bioavailability. The crude extract from SSD is thus used in a number of patented Chinese medicines, and the demand for the crude resource is increasing rapidly today. However, unlike other legume families, the seedling growth proportion of SSD is relatively weak; the fruit drops off too early, resulting in poor reproductive ability [2]. SSD usually takes 7 years to develop and yield effective drugs, which can then be used as medicines. Over the past decades, SSD has been recognized as an endangered plant species and has been documented on the Red List of Biodiversity in China, as a consequence of its low growth rate and man-made activities, including deforestation and overexploitation [3]. Furthermore, the market demand for SSD is massive, and the wild resources can no longer meet these mounting requirements. Thus, the Ministry of Ecology and Environment encourages the artificial planting of SSD in highland areas of Xishuangbanna, southwest Yunnan, China. This area is characterized by a distinct climate, sporadic rainy events, and dry seasons, which can be used in order to cultivate surplus numbers of SSD.

Figure 1. The dry stem of SSD.

2. Phytochemistry

SSD comprises distinguished polyphenol compounds, including flavonoids, carotenoids, chalcone, coumestans, lignans, and phenolic acids [4]. The phenolic contents of SSD are relatively higher than that of many other medicinal plants [5][6][7]. Several analytical methods have been used to isolate, purify, and characterize the chemical compounds from SSD [8][9][10][11]. Several bioactive chemicals in SSD can be identified by HPLC chromatographic fingerprinting. The polyamide column chromatography method is also used to separate polyphenols in SSD. This is followed by the HPLC method to determine the flavonoid components, which enables quality control assurance in identifying the compounds in SSD [12]. Ultraviolet spectrophotometry applications in vanillin–acid assays and the aluminum nitrate colorimetric method are also employed to determine the content of condensed tannins and total flavonoids in SSD [13][14][15][16].

About 57 chemical ingredients in the SSD stem were isolated by the effective method of combining Ultra-Fast Liquid Chromatography and Tandem Mass Spectrometry. The content of chemicals detected in the descending order was isoflavones, flavanols, phenolic acid esters, terpenoids, lignans, and coumarins. Among a pool of chemicals, (-)-gallocatechin, (-)-catechin, (-)-epicatechin, biochanin A, (-)-epiafzelechin, 4,7,2′-trihydroxy-4′-methoxyisoflavanol, β-sitosterol, dihydrocajanin, and maackiain exhibited the highest concentrations in crude extracts [17]. Different analytical techniques can be used to determine the content of bioactive compounds that distinguish the quality and quantity of SSD. A total of 16 compounds were found in SSD as identified by HPLC-MS/MS [18]. Four active flavonoids, viz., protocatechuic acid, catechin, gallocatechin, and formononetin, were determined in the plasma of rats by a UPLC-MS/MSn method, and these active principles were successfully employed in a pharmacokinetics investigation employed after the oral administration of SSD [19]. Diversified compounds from SSD have been identified and classified based on the class, and subclass.

3. Pharmacological Activity of SSD

3.1. Antioxidant Activity

SSD has excellent antioxidant properties due to its phenolic acids, flavonoids, lignans, tannins, and other polyphenols. Principally, catechin, epicatechin, formononetin, gallic acid, syringic acid, and vanillic acid have been described to account for the antioxidant properties of SSD. These antioxidant activities are principally due to their reducing capacity, in which they scavenge or neutralize free radicals and reduce lipid peroxidation [20][21][22]. Furthermore, polyphenols actively involve the chelation of metal ions, causing weakening/cessation of the oxidative process [23][24]. SSD-containing polyphenols exhibited the highest total antioxidant capacity as determined by several in vitro techniques [7][23][25][26][27][28]. SSD exhibited noteworthy neuroprotective benefits on hydrogen peroxide (H_2O_2)-induced cell death in PC12 cells [5]. Porcine circovirus 2 (PCV2) infection generates a large volume of nitric oxide (NO), reactive oxygen species (ROS), glutathione disulfide (GSSG), xanthine oxidase (XOD), and myeloperoxidase (MPO) activities, and a severe decrease in

the activities of GSH and SOD in RAW264.7 cells. However, treatment with SSD attenuates PCV2-induced oxidative stress markers and enhances the actions of the antioxidant enzymes [26][27]. Animal studies have also confirmed that SSD possesses the highest antioxidant capacity, as determined by various biochemical parameters including thiobarbituric acid reactive substances (TBARS), catalase (CAT), hydroperoxides, glutathione (GSH), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPx) [26][27].

3.2. Antidiabetic Activity

Diabetes is connected with the risk for oxidative damage that may cause protein glycation in tissues and the oxidation of glucose, generating free radicals such as hydroxyl radicals, hydrogen peroxides, and protein-reactive ketoaldehydes, along with a consequential increase in lipid peroxidation, which results in DNA damage [29][30]. Additionally, the generation of advanced glycation end-products triggers NF-κB and its several downstream target genes, resulting in a high amount of NO production that is thought to be a stimulator of β-cell destruction in the pancreas [31]. Therefore, antioxidants can counter oxidative stress and have favorable implications for the management of diabetes. Studies have reported that SSD has a potential antioxidant and antidiabetic effect against glucotoxicity and STZ-induced diabetes in animal models [32][33]. Previously, in vitro and rodent model studies have shown that extract of SSD significantly increased glucose uptake and increased GLUT4 expression in skeletal muscle through the downstream regulation of AKT and AMPK pathways in C2C12 cells and STZ-exposed diabetic mice. Besides, SSD significantly improved the antioxidant enzymes and mitigated the expression of gluconeogenic enzymes in the STZ-induced Type 2 diabetes mice model [32].

3.3. Anti-Inflammatory Activity

It has been discovered that a number of inflammatory reactions are triggered by a mucosal injury, microbial infection, and oxidative stress, leading to the identification of pathophysiological mechanisms that are to detect noxious agents infecting the host, such as lipopolysaccharide (LPS) [30][34]. LPS is an antigen of the bacterial cell, which induces macrophage activation, causing excessive NO synthesis, inflammatory mediators, prostaglandins, TNF-α, and several pro-inflammatory cytokines [35][36]. A possible method for treating inflammatory diseases is to attenuate the production of these inflammatory mediators.

With mounting evidence from in vitro and animal studies, SSD exhibits a promising efficacy as an anti-inflammatory drug. Aqueous and ethanolic extracts of SSD show a significant effect on the inhibition of NO and TNF-α production [7]. More than dozens of bioactive compounds isolated from SSD such as (+)-epipinoresinol, (+)-pinoresinol, 2,6-dimethoxy-1,4-benzoquinone, 3-methoxydaidzein, 8-O-methylretusin, biochanin A, butesuperin A, calycosin, daidzin, formononetin, genistein, isoliquiritigenin, liquiritigenin, maackiain, odoratin, and ononin have been screened for their anti-inflammatory efficacy [37]. Furthermore, Spasuberol A, B, and C have also been identified and characterized from SSD and have been reported as anti-inflammatory agents, which was determined by decreasing NO production in RAW264.7 macrophages stimulated by LPS [38].

3.4. Neuroprotective Activity

Apoptotic cell death in neuronal cells plays a key role in controlling several neurological disorders, viz., Parkinsonism, Alzheimer's, Huntington's, and ischemic stroke. Studies show SSD has therapeutic potential for neurological-disorder-related cell death or ischemic stroke [39].

3.5. Hematopoietic Activity

SSD has been employed to heal blood stasis syndrome by impeding platelet aggregation, pro-angiogenesis, and provoking hematopoiesis since time immemorial. By stimulating hematopoiesis and erythroid cells, catechin in SSD may inhibit the disease-related blood stasis syndrome [40][41]. Clinical studies have further established that a decoction of SSD stimulates hematopoiesis and restores the marrow microenvironment [42][43].

3.6. Antimicrobial Activity

Various extracts of SSD have shown excellent antimicrobial properties. As of now, SSD has been recognized to prevent the growth of bacteria (Staphylococcus aureus and S. mutans) and viruses (HIV Type 1, HCV, Coxsackievirus B3) [44][45][46]. Among its crude extracts, 7-hydroxy-6-methoxyflavanone, EGCG, EGC, and formononetin have been reported to have notable anti-microbial properties. Methanolic extracts of SSD showed significant inhibition (>90%) effects on HIV Type 1 [44] and inhibit all three stages of the virus's life cycle in Coxsackievirus B3 [45], which has been determined through the lessening of infection titers in the tainted cardiac cells of rats.

4. Anticancer Activity of SSD

There has been evidence that SSD plays a positive role in the treatment of leiomyoma, breast cancer, glioblastoma, and leukemia. The anti-breast cancer effect of SSD has attracted the most attention among researchers. SSD has potential anticancer effects through apoptosis and pyroptosis induction, cell-cycle arrest, estrogen receptor hypoactivity, proteasome inhibition, anti-mutation, and ROS regulation [4][15][47][48][49][50][51]. Surprisingly, unlike anti-glioblastoma, SSD's anti-breast cancer and anti-myeloma efficacy are reliant on ROS induction. An earlier clinical cohort study demonstrated that SSD has prospective benefits for patients with acute myeloid leukemia [52]. The chloroform and ethyl acetate subfractions of SSD administration were reported to be potent by preventing leiomyoma and reducing the expression level of TGF-beta receptor 2 [53]. A dose-dependent manner of SSD treatment exerted cytotoxicity in the myeloid-originated hematological cancer cell lines U266 and U937, which upregulated apoptosis-related proteins (PARP, procaspase-3, and Bax) and ER stress-related proteins (p-ATF2 and CHOP). Furthermore, SSD inhibited onco-miRNA (miR657) targeting the ER stress signal pathway [47]. Interestingly, KEGG and GO analysis interpreted that SSD could attenuate metastasis in the lung primarily by mediating oxidative stress, AGE-RAGE signaling, and microRNAs [54][55]. Similarly, Network pharmacology analysis revealed that SSD exhibited an anti-ovarian cancer efficacy by activating the key proteins GSK-3β, Bcl-2, and Bax [56].

Previously, studies found that EGCG, a flavanol in SSD, promotes apoptosis in the head, neck, and colorectal cancer [57][58]. Furthermore, the anticancer effect of gallic acid obtained in SSD was present in lung and prostate cancer [59][60]. Moreover, a lab previously reported a novel function of isoliquiritigenin, the active principle from SSD, as a natural inhibitor of autophagy-related miR-25 that promoted autophagy, chemosensitization, and cell-cycle arrest in drug-resistant MCF-7/ADR BC cells. Thus, isoliquiritigenin acts as a natural autophagy inducer to enhance BC chemosensitivity by targeting ULK1 [61].

The chemical constituents of SSD have massive pharmacological effects and clinical applications. SSD contains the analogue of isoliquiritigenin that is used for the prevention of BC [62]. Cyclization of OH-2′ chalcones or α, β-unsaturated isoliquiritigenin significantly induced cytotoxicity in BC cell lines. Likewise, 3′,4′,5′,4″-tetramethoxychalcone is also known as a promising cytotoxic analogue against BC [62]. Interestingly, 3S)-7-hydroxy-8,2′,4′-trimethoxyisoflavane and (3S)-7-hydroxy-8,2′-dimethoxy-4′,5′-methylenedioxyisoflavane in SSD inhibit cell growth and the viability of MCF-7 cells. Also, sativan extracted from SSD is highly hazardous to both MCF-7, 4T1, and MDA-MB-231 cell lines, indicating that SSD is a potential candidate for the inhibition of TNBC cells [62]. Moreover, sativan, another active compound extracted from SSD, has been described to improve the expression of Bax and reduce the protein expressions of Bcl-2 and PD-L1, inhibiting the invasion and migration of TNBC cells by the upregulation of miR-200c [63].

Methanolic extracts of SSD contain several bioactive compounds, including isoliquiritigenin, genistein, 7-hydroxyflavanone, liquiritigenin, daidzein, medicarpin, and formononetin, which have significant inhibitory activity on the human 20S proteasome [37]. Various proteasome inhibitors exert anti-cancer activity in vivo and significantly induce apoptosis in cancer cells in vitro [21][24][64], and SSD and its active compounds are potential proteasome inhibitors [37]. The prevention of N-nitrosamine-induced DNA damage is a key factor for cancer chemoprevention. It has been reported that SSD containing the bioactive compounds genistein, isoliquiritigenin, medicarpin, and naringenin has antigenotoxic effects by preventing the production of the carcinogenic N-methyl-N-nitrosourea [65]. It seems that flavonoids have hydroxyl radical-scavenging capacity that is connected to their antimutagenic properties [65]. 7,4′-dihydroxy-8,2′,3′-trimethoxyisoflavan extracted from SSD has significant cytotoxicity in preventing human cancer cell lines, viz., HL-60, SMMC-7721, SW-480 MCF-7, and A-549 [66]. The ethanolic extract of SSD contains four novel isoflavones, which exert potential cytotoxicity activities in MCF-7 and MDA-MB-231 BC cell lines [49].

5. Mechanism of Anticancer Activity of SSD

5.1. RAS/Raf/MAPK Signaling

MAPK is an active signaling cascade pathway in BC that encompasses numerous key pathways involving a phosphorylation mechanism that has a primary function in tumorigenesis. Signaling pathways are altered in cancer, leading to an aberrant stimulation of tyrosine kinases, which leads to an increase in RAS/RAF gene expression, providing uncontrolled cell proliferation, survival, invasion, and metastasis—hallmarks of cancer [67]. The downstream cascade signals, RAS/RAF/MAPK, have a key function in cell survival and the development of BC (**Figure 2**). MAPK pathway generally comprises the tumor suppressor gene, p38, ERK 1/2, and JNK 1/2 in normal breast cells [68]. However, activation and upregulation of ERK 1/2 and JNK 1/2 occur in BC cells, which promotes transcription activation of genes related to uncontrolled cell growth [69]. SSD and its active constituents provoke their anti-BC effects by networking and inhibiting the downstream signaling pathways of RAS/RAF/MAPK.

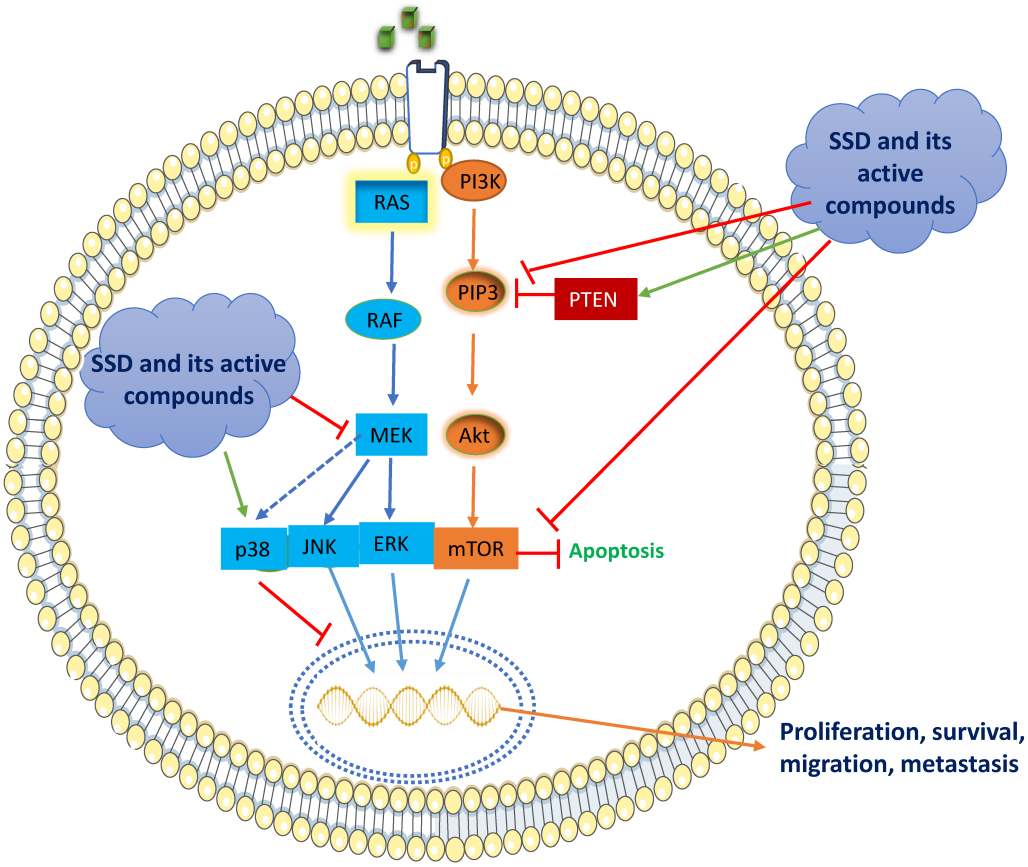

Figure 2. The role of SSD and its active constituents in inhibiting cell proliferation, survival, migration, and metastasis through the RAS/Raf/MAPK and PI3K/Akt/mTOR signaling pathways.

5.2. PI3K/Akt/mTOR Signaling

The remarkable downstream signaling of PI3K/Akt/mTOR is frequently engaged in most of the BC types, accounting for over 70% [70][71][72][73]. In turn, PI3K stimulates the conversion of PIP2 to PIP3, which phosphorylates protein kinase B, Akt, which in turn activates the serine/threonine kinase, mTOR [74][75]. The cascade signaling of PI3K/Akt/mTOR is a crucial key step in the cell cycle, tumor development, and survival [71]. However, the dephosphorylation of PIP3 to PIP2 activates Akt by an enzyme, PTEN, which is a well-recognized tumor-suppressor protein. (**Figure 2**) [72]. Activation of the PI3K/Akt pathway occurs via insulin-like growth factor 1 receptor (IGF-1R), thereby phosphorylating IGF-1R and resulting in the recruitment of Akt [76].

SSD and its active constituents exert their anti-cancer effects by networking with complex transcription factors, PI3K/Akt/mTOR. SSD comprises calycosin daidzein, formononetin, and genistein with a dose range of 80–320 µg/mL that inhibits the protein expressions of PI3K/Akt/mTOR in MCF-7 cells [50]. Resveratrol and genistein in the doses of 0.1, 1, 5, 10, 100, and 1000 nM significantly elevated PTEN expression (PI3K/Akt inhibitor) in MCF-7 and MDA-MB-435 BC cells [77]. The treatment of calycosin (25–100 µM) and formononetin (20–80 µM) inhibited PI3K/Akt signaling in T47D and MCF-7 cells by reducing the activation of IGF-1R protein levels and resulting in the inhibition of Akt phosphorylation. Similarly, the treatment of formononetin (10–40 µM) decreased the expression of p-PI3K/p-Akt in TNBC cells [78]. Another investigation demonstrated that an analog of resveratrol, MR-3 (10, 20 µM), attenuated PI3K/Akt signaling in MCF-7 cells by preventing the phosphorylation of Akt and inhibition of GSK-3β [79]. SSD contains another bioactive compound, baicalein, which is demonstrated to have an anti-BC effect through the downregulation of the expression of p-AKT, p-mTOR, NF-κB, and p-IκB and the upregulation of IκB in MCF-7 and MDA-MB-231 cells [80]. Altogether, SSD and its active constituents inhibit the PI3K/Akt pathway by inhibiting IGF-1R, phosphorylating Akt, and enhancing the inhibitory activity of PTEN.

6. Conclusions

SSD and its active compounds are auspicious medicinal drugs implicated in the treatment of various ailments since time immemorial. SSD comprises distinguished polyphenolic compounds, including flavonoids, chalcone, dihydroflavone, pterocarpan, and phenolic acid. These compounds have potential pharmacological effects, viz., antioxidant, antidiabetic, antimicrobial, hematological, neuroprotective, and anticancer properties. There is significant potential for both in vitro and

in vivo studies to combat BC types. Furthermore, SSDs exert their anti-tumor effects by modulating PI3K/Akt/mTOR and Ras/Raf/MAPK pathways, preventing BC development.

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