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Chinese Journal of Natural Medicines 2017, **15**(4): 0255–0264
Chinese Journal of Natural Medicines

•Review•

Saposhnikoviae divaricata: a phytochemical, pharmacological, and pharmacokinetic review

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Available online 20 Apr., 2017

[ABSTRACT] *Saposhnikoviae divaricata* (Turcz.) Schischk (SD) is a traditional Chinese herb commonly used to treat clinical conditions such as rheumatism and allergic rhinitis. This review article evaluates a collection of works on *in vitro* and biochemical studies of SD. The discourse on the diverse class of chromones and coumarins in SD offers an insight to the pharmacological effects of these bioactive constituents as anti-inflammatory, analgesic, immunoregulatory, antioxidative, and anti-proliferative agents. It is highlighted that there is a structural relationship between the constituents and bioactive activities, which in effect provides a valid reasoning and reaffirm the use of SD in the treatment of the pathologies in Chinese medicine.

[KEY WORDS] *Saposhnikoviae divaricata*; Chinese herbal medicine; Anti-inflammatory; Analgesic; Immunomodulatory; Anti-proliferative

[CLC Number] Q5; R965; R969.1 **[Document code]** A **[Article ID]** 2095-6975(2017)04-0255-10

Introduction

Saposhnikoviae divaricata (Turcz.) Schischk (SD), an umbelliferous genus, is widely found in the northern and northeastern territories of China and cultivated in many other areas such as Anhui, Shanxi, and Gansu provinces. It is known as “Fang Feng” in China, “Bou-hu” in Japan and “Bangpung” in Korea. The Chinese terminology of this herb literally translates “to avert wind”, and the dried roots of this plant is generally applied to treat pathogenic conditions of wind-damp-cold in Chinese medicine (CM). SD is an important component in traditional Chinese medicine clinical practice and commonly used for the treatment of rheumatism, arthralgia, general aches, headaches, stroke, fever, cold, and allergic rhinitis. The Chinese Pharmacopoeia Commission [1] ranked SD as a top-grade herb, and it is described to possess pungent, sweet and slight warm properties. SD has a wide spectrum of use in the CM. Its applications are recorded and well established in the CM classical text the Shen Nong’s *Materia Medica* (*Shen Nong Ben Cao Jing*) which is the oldest pharmaceutical monograph in CM, dating back to Qin-Han dynasty. In the Shen Nong’s *Materia Medica*, SD is graded as a premium-grade herb cited for its multiple effects: immunoprotective capability in warding off cold (also otherwise known as dispelling wind-cold and relieving exterior pathogens in CM), relief of edema-induced pain (expelling dampness from the acupuncture meridians) and anti-spasmogenic activity (expelling wind and relieving convulsion). Similarly, in the Collected Works of Materia Medica (*Ben Cao Hui Yan*), authored by NI Zhu-Mo in the Ming dynasty, a herbal monograph outlined the multiple use of SD for the treatment of arthritis instilled with dampness, joints pain, tetanus, atrophy and flaccidity of muscles, headaches, cold, fevers, cough, adiaphoresis, nasal congestion, pharyngeal dryness syndrome, cerebrovascular accident, early onset of small pox, and anxiety in children. CM clinicians today still use SD for the same pathologies cited. In response to the pressing need to align CM for an evidence-based approach, there is an emergence of scientific research on the bioactive constituents and the therapeutic effects of SD. Therefore, the objective of this review article is to provide a systematic review of phytochemical, pharmacological, and pharmacokinetic perspectives of the experimental studies on SD.

Methods

The following databases were searched from their respective receptions up to January 2016: PubMed; EMBASE;

[Received on] 20-Jun.-2016
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All the authors have no conflicts of interest to declare.

AMED; CINAHL; Cochrane Library; MEDLINE; ScienceDirect; SCOPUS; Web of Science; China Network Knowledge Infrastructure; CQVIP; and Wanfang Data. The keywords used for the literature search included: Fang Feng and its English, botanical and pharmaceutical names. The papers identified from the search were screened according to the criteria laid out in the Australian Regulatory Guidelines for Complementary Medicines and Therapeutic Goods Orders for quantitative and qualitative analysis of herbal medicine by the Australian Therapeutic Goods Administration (TGA). TGA adopts the British Pharmacopoeia and Therapeutic Goods Orders as the official guidelines for testing and acceptance criteria for herbal substances, herbal preparations, and herbal medicinal products, inclusive of traditional herbal medicinal products [2]. TGA also recognizes other international pharmacopoeias for guidelines on assessing and testing botanicals in complementary medicine.

The selection criteria included process controls of the herbal substances, reporting reference standards such as authentication of reference materials and profile chromatograms, and analytical procedures and validation data. Papers in English or Chinese language are considered for this review. Scientific rigors were called upon to determine the chemical markers of herbs through the use of strict parameters in testing, quantitative and qualitative measures of the bioactive components, such as fingerprint spectrum, correlations differentiation, and stability evaluation, reference standards, and toxicological assessments. According to the TGA, reporting referencing standards includes naming the origin of the plant materials and its authentication [2]. A plant specimen voucher number (PSVN) is an upmost requirement for referencing standards in botanical testing [3]. It enables traceability of the plant material, allows access to the storage of crude plant for researchers who seek to cross verify data, further scientific investigations or commercial purposes, should there be discrepancies in the drug products produced from the plant source. Essentially, a lack of PSVN essentially thwarts scientific research from the onset and may cause legal repercussions if used for commercial purposes. Attempts to compare bioactive yield outcomes of these studies was unfeasible in this review, owing to differences in extraction weights and yield units used in the papers such as relative standard deviation, microliters or milligrams per liters. The scientific process of reporting chemical profiling by spectroscopic and/or chromatographic fingerprint should include calibration curves that depict the peak area ratio and the percentage of relative abundance of the bioactive compounds yielded from the plant material in study.

Results

A total 135 papers were identified through the literature search. Among them, 10 *in vitro* studies, 25 phytochemistry studies, and 9 phytochemistry with *in vitro* studies were considered for screening after 86 non-*Saposhnikoviae divaricata* and 5 non-phytochemistry related papers were excluded. Phytochemistry papers were further screened according to the selection criteria and 21 papers were excluded due to non-PSVN (n = 17), different reporting standards of measure standard (n = 2), standard procedures of reporting chromatographic profile not followed (n = 1) and study on soil element of SD (n = 1). As a result, 4 SD phytochemical studies only [4-7] adhered to the scientific rigors that ensure the integrity in validation and robustness in quantitative outcomes. Among 9 phytochemistry papers with *in vitro* studies [8-16], 5 [11, 13-16] were selected based on their relevance, but the phytochemistry component did not adhere to the selection criteria. A total of 10 *in vitro* studies only were included in this review [17-26]. Fig. 1 illustrates the study selection process.

Bioactive compounds

Crude plant of SD contains chromones, coumarins, polyacetylenes, and acid esters. A summary of these bioactive compounds of SD reported in the included studies are shown in Table 1. The crude dried roots of SD contain chromones and coumarins, which are both heterocyclic compounds, derived from the flavonoids. Chromone is a class of oxygen-containing heterocyclic compound with a benzo annelated γ-pyrone ring, belonging to a subclass of the flavonoids known as flavone [10]; while coumarin, a benzopyrone, structurally consists of a benzene ring fused to a pyrone ring. A total of 9 chromones are identified in the included studies. The 5 main chromones, prim-*O*-glucosylcimifugin (GC), 4′-*O*-*β*-D-glucosyl-5- *O*-methylvisamminol (GV), cimifugin (C), Sec-*O*-glucosylhamaudol (GH), and 5-*O*-methylvisamminol (MV), are abundant in SD, in particular GC and GV. These chromones are discovered to exhibit strong pharmacological activities in attenuating inflammatory [4], scavenging free radical [20], and inhibiting pain [8] in *in vitro* studies.

Aside from the chromones, a total of 22 coumarins are identified in SD from the included studies. New discoveries of coumarins in SD have also emerged over recent years. Three new coumarin compounds are discovered to have effects against porcine epidemic diarrhea virus (PEDV): divaricoumarin A, divaricoumarin B, and divaricoumarin, with molecular formulae being $C_{25}H_{32}O_{12}$, $C_{25}H_{30}O_{12}$, and $C_{25}H_{30}O_{12}$, respectively [10] (Fig. 2). Among the SD coumarins tested, Praeruptorin B is found to have the strongest effect against PEDV in Vero cells. *In vitro* results reveal Praeruptorin B is able to inhibit viral replication at the stage of protein syntheses. Although PEDV is essentially a viral infection in pigs, this coronavirus stems from the same family of the human coronaviruses, such as severe acute respiratory syndrome and Middle East respiratory syndrome [10]. Praeruptorin B, a coumarin from SD, could potentially offer a novel anti-viral drug research for the intractable human coronavirus diseases. In addition, phenylpropanoid fatty acid ester, defined chemically as (±)-2-hydroxy-3-(4-hydroxy-3- methoxyphenyl)-3-methoxypropyl nervonic acid ester and divaricatol are discovered to inhibit nitric oxide (NO) in lipopolysaccharide (LPS)-induced mouse

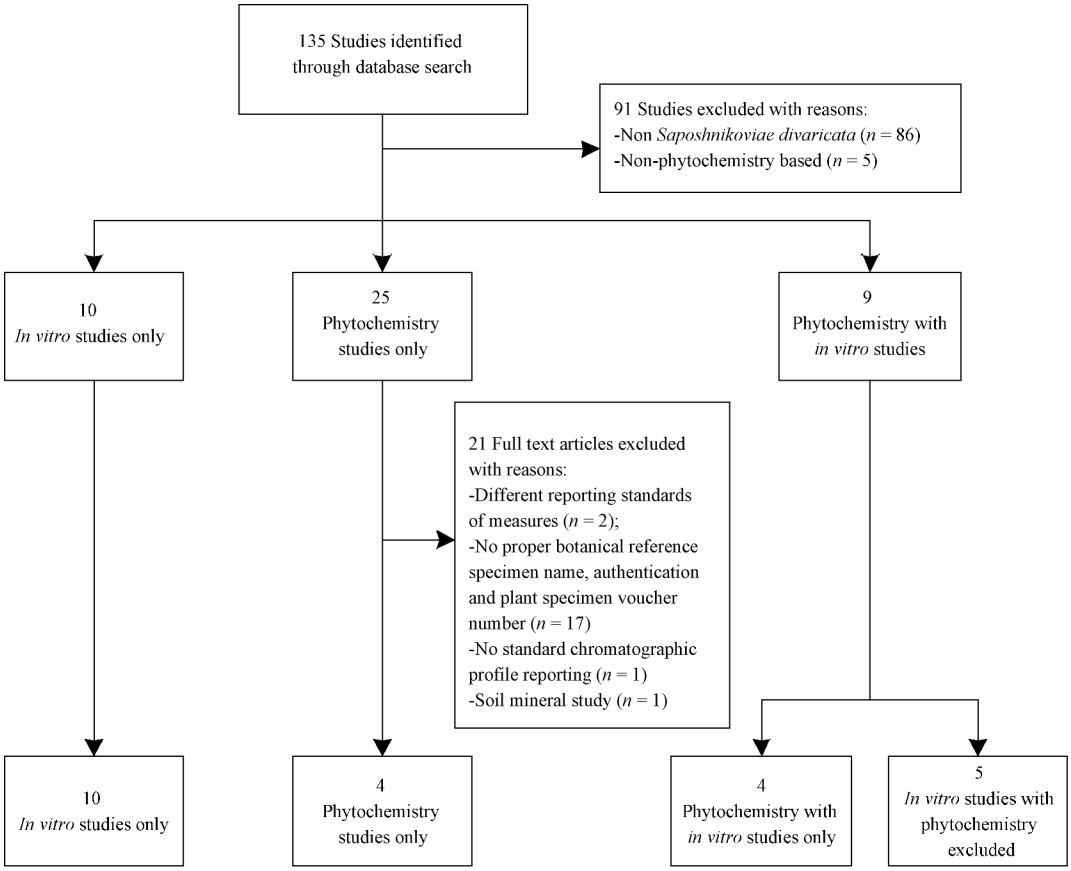


Fig. 1 *Saposhnikoviae divaricata* study selection flowchart

Table 1 Summary of chemical constituents isolated from *Saposhnikoviae divaricata*

Compound derivatives	Chemical compounds	Methods	References
Chromones	Hamaduol (Linear dihydropyranochromone)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
		^{1}H-^{13}C NMR/CC/TLC	[8]
	Ledebouriellol (Linear dihydropyranochromone)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
		^{1}H-^{13}C NMR/CC/TLC	[8]
	Cimifugin (Linear dihydrofurochromone)	HPLC-ESI-MS	[6]
		^{1}H-^{13}C NMR/CC/TLC	[8]
		UAE-RSM/HPLC	[7]
	Sec-*O*-*β*-D-glucosylhamaudol (Linear dihydropyranochromone)	^{1}H-^{13}C NMR/HPLC/ESIMS	[5]
	Sec-*O*-glucosylhamaudol	HPLC-ESI-MS	[6]
		^{1}H-^{13}C NMR/CC/TLC	[8]
		UAE-RSM/HPLC	[7]
	4'-*O*-beta-D-glucosyl-5-*O*-methylvisamminol (Linear dihydrofurochromone)	HPLC/LLOQ/LC-ESI-MS	[9]
		HPLC-ESI-MS	[6]
	Prim-*O*-glucosylcimifugin (Linear dihydrofurochromone)	HPLC/LLOQ/LC-ESI-MS	[9]
		HPLC-ESI-MS	[6]
		UAE-RSM/HPLC	[7]
	5-*O*-methylvisamminol (Linear dihydrofurochromone)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
		HPLC-ESI-MS	[6]
		UAE-RSM/BBD/HPLC	[9]
			[7]
	Divaricatol (Linear dihydropyranchromone)	^{1}H-^{13}C NMR/CC/TLC	[4]
		CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[8]

Continued

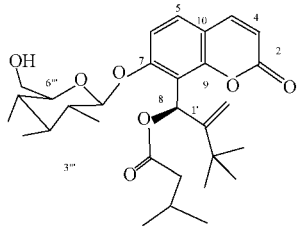
Compound derivatives	Chemical compounds	Methods	References
Coumarins	Anomalin (Pyranocoumarin)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
	Scopoletin (Furanocoumarin)	^{1}H-^{13}C NMR/CC/TLC	[8]
	Marmesin (Linear Furanocoumarin)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
	Methoxy-8-(3-hydroxymethyl-but-2-enyloxy)-psoralen (Furanocoumarin)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
	Byakangelicin (Furanocoumarin)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
Coumarins	Isofraxidin (Simple coumarin)	^{1}H-^{13}C NMR/CC/TLC	[8]
	Fraxidin (Simple coumarin)	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
		CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
		^{1}H-^{13}C NMR/CC/TLC	[8]
	Praeruptorin F (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	Cis-3'-isovaleryl-4'-acetylkhellactone (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	Praeruptorin B (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	Cis-3', 4'-disenecioylkhellactone (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	Cis-3'-isovaleryl-4'-senecioylkhellactone (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	(−)-*cis*-khellactone (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	Oxypeucedanin hydrate (Furanocoumarin)	CC/NMR/ECD/PCR	[10]
	Decursinol (Pyranocoumarin)	CC/NMR/ECD/PCR	[10]
	Umbelliferone (Simple coumarin)	CC/NMR/ECD/PCR	[10]
	Divaricoumarin A	HPLC/ECD/NMR	[10]
	Divaricourmarin B	HPLC/ECD/NMR	[10]
	Divaricoumarin C	HPLC/ECD/NMR	[10]
	Glycerol monolinoleate	^{1}H-^{13}C NMR/CC/TLC	[8]
	Glycerol monooleate	^{1}H-^{13}C NMR/CC/TLC	[8]
	(3'*S*)-hydroxydeltoin	^{1}H-^{13}C NMR/CC/TLC	[8]
Diphenol	Melanochrome	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
Acid esters	(±)-2-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropyl nervonic acid ester	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
	Lindiol	CC/HPLC/YMCC/^{1}H-^{13}C NMR-MS	[4]
Polyacetylene	Panaxynol	^{1}H-^{13}C NMR/CC/TLC	[8]

macrophages RAW 264.7 [4]. It is noteworthy that the diversity of these SD bioactive compounds could offer pharmacological scaffold for developing new agents with anti-inflammatory, antipyretic, anti-viral, anti-oxidant, and analgesic effects.

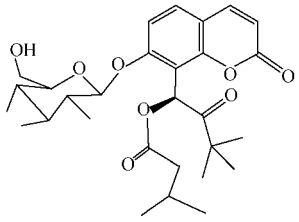
Pharmacological effects and applications

Analgesic and antinociceptive effects

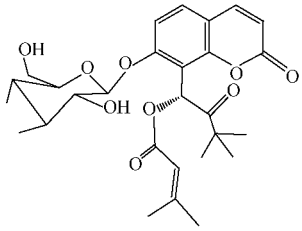
The analgesic effects of SD are mainly due to C, GH (chromones) and anomalin (coumarin), while other chromones such as GV, GC, and MV are found to exert no significant analgesic effects. Hamaudol with its algycone portion and ledebouriellol and divaricatol (both pyranochromones) increased pain threshold, by imparting an opioidergic effect experienced in mice when administered with a high dose of GH [8]. Significant writhing inhibition was observed, but no significant hypothermic effect was detected on the pyrexic mice even at a higher dose of 160 mg·kg^{-1}, and writhing inhibitory effect was easily reversed by naloxone injection [8]. Evidence suggested that the SD analgesic effects were acting on an opioid receptor in the CNS and its pathway, not on the PNS pain-associated inflammation generally produced on the peripheral sensory nerve. These analgesic effects were also detected in a pain threshold tail writhing test with intramuscular injection of GC and GV in murine models at doses of 25, 50 and 100 mg·kg^{-1}. Pain deterrent at higher doses were prolonged with a considerable reduction of spasmodic pain were observed in the models [26]. Anomalin is particularly effective against hyperalgesia and allodynia, exerting strong anti-nociceptive effects after 4 hour at 50 mg·kg^{-1}, inhibiting carrageenan-induced hyperalgesia [19]. The anti-nociceptive



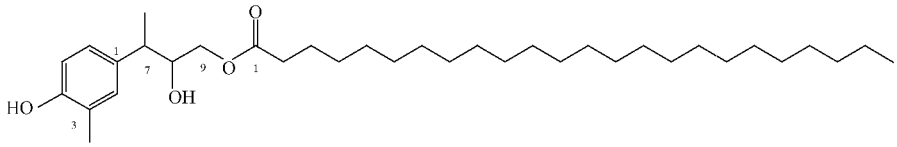
Compound 1 Divaricoumarin A [10]



Compound 2 Divaricoumarin B [10]



Compound 3 Divaricoumarin C [10]



Compound 4 Phenylpropanoid fatty acid ester [4]

Fig. 2 Chemical structures of four new chemical constituents from *Saposhnikoviae divaricata*

effects for acute model persisted for 6 hours, after 2 hours of treatment, while the effects lasted for 5 days for the chronic models.

Anti-inflammatory effects

GC, C, MV, and anomalin appear to exert considerable inhibition in major inflammatory pathways, namely nuclear factor (NF)-κB, mitogen activated protein kinases (MAPKs), cAMP response element-binding protein (CREB), and nitric oxide production. Anti-inflammatory effects of SD chromones were revealed in arthritic rats, including significant decline in the arthritis score for pain and swelling, with a reduction of the inflammatory cytokines levels, although suppression of the tumor necrosis factor (TNF) α and IL-1β was only observed in rats treated with higher dose of SD [21]. A key indicator in inflammation, prostaglandin$_2$ (PGE_2), was also decreased in both sera and joints. Treatment of SD suppressed the activation of NF-κB binding to its DNA sequences. Deactivation of NF-κB binding was facilitated by the suppressions of signal-transduction inflammation induced by TNFα-mediated phosphorylation of ERK, JNK, and p38, which is amplified in both collagen-induced arthritic rats and human fibroblast-like synoviocytes (3.2, 2.8, and 2.6 fold, respectively) [21]. The inhibition of MAPKs phosphorylated -ERK, JNK and p38 was due to the action of GC. Among all the MAPK subtypes, p-JNK was discovered to be most effectively suppressed. The same GC inhibitory effects on ERK1/2, JNK and p38 were detected in LPS-induced inflammation in severe acute lung injury and no cytotoxic effect with the 50% lethal dose (LD_{50}) of SD-GC from 12.5 to 100 mg·L^{-1} [16].

Interestingly, although GC is an inhibitor of NO and iNOS production [16], both C and MV have stronger effects than GC [14]. SD chromones and anomalin are highly effective against acute and chronic inflammation. Obvious anti-pyretic effects in acute carrageenan paw swelling of rats are associated with SD chromones [18] as well as anomalin [21], and similar inhibition of chronic complete Freund's adjuvant (CFA)-induced edema, with lesions being significantly reduced [18]. Both urine HPLC-MS analysis and titre levels in CFA models indicated that GC and GV were mainly responsible for the anti-inflammatory effects in SD. GC, in particular, counteracted the LPS-induced inflammation in severe pulmonary inflammatory disorder, manifested in acute respiratory distress syndrome [16, 18]. *In vivo* and *in vitro* results have demonstrated GC attenuated LPS-induced cytokine levels; TNF-α, IL-1β and IL-6 were far lower in the GC group than that in the control group [14]. Anomalin-mediated inhibitions on iNOS, COX-2, TNF-α and IL6 deactivated the transcriptional onset of NF-κB in LPS-induced RAW 264.7 cells [17]. Cytoplasmic expression of IκBα was drastically reduced, along with subunits of NF-κB, p50, p65 and c-rel being translocated; these were marked indications of anomalin obliterating NF-κB-DNA binding activities. Although anomalin was able to interrupt protein synthesis of IκBα and phosphorylation, there was no inhibitory effect on the protein initiating factor, eIG2α phosphorylation. Anomalin is effective against hyperalgesia and allodynia associated inflammation. Elevated MARK-ERK and p38 signaling and nuclei translocation are precursors to activation of the CREB pathway [27]. Anomalin, besides its ability to deactivate the NF-κB pathway, is also effective in deactivating CREB pathway, which is generally associated with pro-nociceptive mediators that induce prolonged pain in chronic inflammation. MARK-ERK and p-38 expressions are exponentially reduced, abolishing the CREB-DNA binding activity [19].

Similarly, GV, GC, C and GH are matrix metalloproteases (MMP) inhibitors [11]. MMP 2 and MMP-9 are responsible for clearing collagens fragments generated by collagenases in tissues repair and remodeling. Enhancement of both MMP-2 and MMP-9 activities induced by the IL8 secretion are inherent biomarkers of cancer [28]. In another study, GV, GC, C and GH were shown to have concentra-

tion-dependent inhibitory effects in *in vitro* MMP assay, with IC_{50} values being 15.6, 108.87, 313.25, and 344.4 μmol·L^{-1}, respectively [11] and GV being the strongest inhibitor of MMP-2. When analyzed among other herbs studied in a herbal formula, although SD was discovered to inhibit MMP-1, MMP-2, and MMP-9 activities, it also promoted TNF *α*-induced -IL-8 secretion, which indirectly promoted angiogenesis [22].

Anti-oxidant and anti-proliferative effects

Four cancer cell lines, *i.e*., human mammary adenocarcinoma MCF7 and MDA-MB-468, leukemia K562, and myelomonocytic HL60 cells, and murine RAW 264.7 macrophage/monocyte cells were tested *in vitro* with camptothecin (CAM), paclitaxel (PTX), and in combination with SD [20]. Among them, HDL60 was most susceptible to SD ethanol extract (1 g/10 mL) while MCF7 the least. The estimated 50% inhibitory concentration (IC_{50}) were at 1/300, 1/400, 1/250, and 1/600 dilutions for K562, HL60, MCF7 and MDA-MB-468 cells, respectively [20]. Synergistic effects with the combined treatment of chemotherapeutic agents, CAM or PTX, on the four cancer cells have been demonstrated; SD extracts effectively reduced the IC_{50} values of CAM in K562 and HL60 cells and that of PTX in MCF7 and MDA-MB-468 cells (Table 2), compared with cells treated with chemotherapeutic agents alone. Co-administration of SD with a lower concentration of chemotherapeutic agents could effectively achieve the same anti-proliferative effects as compared to a high cytotoxic concentration of CAM or PTX.

Table 2 IC_{50} values and percentages (%) reduction of CAM or PTX and combined cell proliferation effects with *Saposhnikoviae divaricata* extract [20]

Cell Lines	K562	HL60	MCF7	MDA-MB-468
IC_{50} and % reduction	SD 1/1 000 dilution + CAM 0.097 ± 0.014 (75%) SD1/500 dilution + CAM 0.13 ± 0.03 (54%)	SD 1/2 000 dilution + CAM 0.064 ± 0.027 (78.5%)	SD 1/500 dilution + PTX 4.93 ± 0.39 (17.8%) SD1/300 dilution + PTX 1.53 ± 0.54 (74.5%)	SD 1/300 dilution + PTX 2.32 ± 0.24 (51.9 %)

CAM: camptothecin; IC_{50}: 50% inhibitory concentration; PTX: paclitaxel

However, this antagonistic effect on cell proliferation was highly dependent on the concentrations of SD extracts and the chemotherapeutic agents. Panaxynol, an active component of SD root was discovered to suppress cell proliferation in K562, Raji, Wish, HeLa, Calu-1 and Vero cells by 30.0% ± 4.1%, 34.0% ± 5.6%, 19.4% ± 3.2%, 32.0% ± 8.5%, 14.5% ± 16.8% and 8.9% ± 3.2%, respectively, at 25 μmol·L^{-1} [23]. Complete inhibitory effects were observed *in vitro* at 100 μmol·L^{-1} with evidence of cell cycle arrest from G_0/G_1 phases to S and G_2/M phases. Remarkably, this inhibitory effect of Panaxynol on the tumor cells proliferation and the cell arrest cycle could possibly suggest an impairment of cyclin E mRNA level. Laser densitometry analysis confirmed a reduction of the ratio of cyclin E mRNA to *β*-actin mRNA *in vitro* [23].

Immunoregulatory effects

The immunoregulatory effects of SD polysaccharides were tested using spleen proliferation index and spleen index as well as the macrophage and its phagocytic rate, showing marked difference in spleen proliferation index [24], but no significant difference in the spleen index. Obvious increases in the phagocytic rate and macrophage index in mice coincided with the increased doses (Table 3). Lymphocyte subsets ratio for CD3+CD4 increased from 27.28% ± 2.30% (250 mg·kg^{-1} dose) to 45.82% ± 1.54% (1 000 mg·kg^{-1}), while CD3+CD8+ was significantly high at 17.44% ± 1.78% (250 mg·kg^{-1}) but decreased by 13.22% ± 1.34% (1 000 mg·kg^{-1}). Significant differences were detected in subsets CD4+CD8+ ratio from 1.58% ± 0.18 % (250 mg·kg^{-1}) to 3.49% ± 0.29% (1 000 mg·kg^{-1}), as well as CD19+ from 10.42% ± 2.40% (250 mg·kg^{-1}) to 15.15% ± 2.32% (1 000 mg·kg^{-1}) [24].

Pharmacokinetics

The bioavailability of drugs is largely influenced by solubility and human intestinal permeability [29]. A study evaluated SD with microsomal testosterone 6*β*-hydroxylation as an index marker for CYP3A4 activity in response to SD furanocoumarins in human liver [12]. Samples of SD tested had no significant CYP3A4 inhibition. Compounds anomalin, 5-methoxy-8-hydroxy psoralen, decursin and decursinol angelate, C and GH were found to be well-absorbed using the Cacao-2 monolayer model at concentration of 50 μmol·L^{-1} [25]. These SD compounds were deemed to be well absorbed, with 3'-*O*-angeloylhamaudol and GH being moderate absorbent compounds. Increased linear kinetic curves depicted influx measurement across Cacao-2 monolayer indicated a passive diffusion mechanism for SD compounds, except for GH and 3'-*O*-angeloylhamaudol. Both compounds possessed a comparably smaller permeability magnitude in the first-pass metabolism. All the bioactive constituents of SD were easily absorbed in the first-pass metabolism and did not seem to manifest any interference with CYP3A4.

Table 3 Effects of *Saposhnikoviae divaricata* polysaccharides on phagocytic rate and macrophage index in mice [24]

	Blank control	SD polysaccharides 250 mg·kg^{-1}	SD polysaccharides 500 mg·kg^{-1}	SD polysaccharides 1 000 mg·kg^{-1}
Phagocytic rate %	25.27 ± 8.66	30.06 ± 7.61	46.36 ± 10.21	52.68 ± 12.35
Macrophage index	0.61 ± 0.11	0.65 ± 0.12	0.74 ± 0.07	0.91 ± 0.08

An interesting mechanism in the absorption and elimination of two SD main chromones GC and C has been discovered. The conversion of GC to C was marked by the presence of higher concentration in the blood after absorption. This was indicative that GC is essentially a prodrug of C. GC exhibited low bioavailability, with evidence in its rapid elimination in the concentration-time curve, with $t_{1/2}$ being 1.02 ± 0.034 h after oral administration [13]. A delayed double peak occurred in mass spectrometry after the administration of the SD extract at 1.55 and 6.40 h with an increased Area-Under-Curve. Both absorption and elimination of cimifugin were longer as a combined SD extract than a single administration of C monomer solution alone [13].

Discussion

The collection of works reviewed herein illustrates the diverse bioactive properties of SD associated with active pharmacological actions *in vitro*. Clinical applications of SD demonstrated in these experimental studies underscored the potency of the bioactive compounds of SD and its pharmacological effects. Modern day diseases challenge medical researchers to look beyond the synthetic drugs and consider the bioactive compounds in natural products.

SD chromones could provide analgesic, antinociceptive and anti-inflammatory effects in neuropathic pain management. Its capability to downregulate the mediators and proinflammatory proteins in the process of signal transduction cascade, in particular, NF-κB and MAPKs inflammatory pathways. Both pathways are constituted in rheumatoid arthritis (RA) manifesting pathological symptoms such as pannus formation, synovitis and cartilage damage, and bone erosion [30]. Proinflammatory cytokines such as TNF-α and IL-1β, chemokines, bacterial and viral products, UV radiation and free radicals could activate inhibitory protein IκB, inducing the phosphorylation, ubiquitination and degradation of IκB subunits by the IκB kinase (IKK) complex [31]. Although *in vitro* evidence has demonstrated that SD chromones deactivate proinflammatory cytokines TNF-α and IL-1β in RA *via* a disruption to MAPKs subtypes ERK, JNK and p38 phosphorylation, thus effectively interrupting the canonical pathway of NF-κB, the molecular mechanism by which the SD chromones deactivate ERK, JNK, and p38 has yet to be elucidated.

The course of inflammation is often accompanied by tissue remodeling and repair, which involve MMPs, which are a group of zinc dependent endopeptidases, involved in the degradation and removal of extracellular matrix molecules from tissues. These tissues are responsible for the regeneration of the extracellular matrix of skin manifested in wound healing, tissue repair, and remodeling in response to injury such as myocardial injury, atheroma, arthritis, cancer, and chronic tissue ulcer [32]. One hallmark of cancer proliferation is the result of IL-8 signaling which promotes angiogenesis and metastasis of malignant tumors. Anti-cancer drug designs for MMP inhibitors are aimed at several targets, namely chelating zinc ion at MMP's active sites, inhibiting MMP's enzymatic activity as well as synthesis, and the latest utilizing bisphosphates and D-tryptophan derivatives to inhibit gelatinases [32]. Findings on SD capability to inhibit MMP-2 and MMP-9, along with the discovery that SD indirectly promoted angiogenesis *via* production of IL-8 were conflicting [22], had casted questions as to whether effects of SD could be diminished or adulterated when used in a combined herb formula. Nonetheless, merits of GV in SD reflected its high affinity to enzyme MMP-2 and SD chromones' inhibitory effects on IL-8 expressions in the signaling cascade that could potentially halt the tumorigenesis process and curtail MMP-9 activities vigorously. MMP-9 has a distinct role in tumor angiogenesis because it regulates vascular endothelial growth factor (VEGF), the most potent inducer of tumor angiogenesis and a major therapeutic target [33]. These chromones could potentially be used as a therapeutic intervention in targeting MMP-9 and VEGF downstream effectors and targets, although further study on the mechanisms responsible for the inhibitory effects of SD chromones on MMP-2 and MMP-9 is still needed.

Immunomodulation of proinflammatory state in innate and adaptive immunity can easily be reversed into a stabilized anti-inflammatory mode. SD polysaccharides could be used as an immunomodulation agent to treat autoimmune diseases commonly characterized with chronic proinflammatory. Plant–derived polysaccharides are posited to exert an immunomodulatory effect by the binding to specific receptors on immune cells in the gut-associated lymphoid tissues and activate intracellular signals [34]. The T cell receptors in T cell activation indicate the 'status' of the adaptive immunity. Low ratios of $CD4^+$and $CD8^+$ are essentially associated with the immunosenescence of the immune system and is also a distinct feature of viral infection such as human immunodeficiency virus, and tuberculosis infections [35]. Lymphocyte subsets CD3+CD4+ and CD3+ CD8+ mediate immune response in B cells differentiation and macrophage activation. A recovery of these phenotype biomarkers elicited an immune reconstitution in immunodeficiencies could be facilitated by the SD polysaccharides. The basis of this biologic activity of polysaccharides could possibly lie in the binding of its monosaccharide composition of rhamnose, mannose, glucose, and galactose; promoting sialylated changes on glycoproteins or otherwise reverting to a normalized anti-inflammatory mode in IgG, while an absence of it would promote pro-inflammatory effector function [34]. The potential role of SD polysaccharides could offer promising outcome for chronic persistent infections, in particular, with the elucidation of SD polysaccharides based on chemical-structural relationship to the receptors. This would provide a rational basis for clinical research to develop the novel drugs for autoimmune diseases.

The concept of co-administration with complementary medicine such as herbal formulae is not a novel practice in CM, although it needs to be substantiated with scientific findings. Co-administration with SD extracts and chemotherapeutics exhibited no cytotoxicity or side effects *in vivo*. Coumarins and its derivatives such as 7, 3 hydroxy coumarins and psoralen are known to possess anti-oxidant properties against human cancer cell lines [36-38]; it appears that the number of hydroxyl groups on the coumarin correlates with the role as a suppressor of reactive oxygenated species [39]. Structurally, natural products with phenolic groups with at least two polar groups in benzene ring at positions 6, 7 or 7, 8 are postulated to possess apoptotic properties while presence of hydroxyl groups at positions 5, 6, 7, and 8 of aromatic nucleus exhibit anti-inflammatory properties of hydroxycoumarins [40]. As part of the umbelliferae family, SD contains many natural coumarins and some of the SD pyranocoumarins substitution pattern indicated for praeruptorin F, *cis*-3'-isovaleryl-4'- acetylkhellactone, praeruptorin B, *cis*-3', 4'-disenecioylkhellactone, *cis*-3'-isovaleryl-4'-senecioylkhellactone, (−)-*cis*-khellactone were at C-8/C-1'/C-3'/0/C-7, with divaricoumarins at C7 and C8 [10]. Moreover, strong synergistic interactions were also featured when pyranocoumarins were combined with common anti-tumor drugs such as vincristine, doxorubicin, and PTX [41]. These structural properties of SD engender anti-neoplastic attributes that could pose as an anti-cancer drug or an adjuvant in mainstream therapeutics with lowered cytotoxicity for chemotherapeutics. In view of this recommendation, future clinical research needs to pave the way to understand the mechanism of combining natural products as mainstream medicine.

The bioavailability of drugs and herbs alike, is subjected to absorption, distribution, metabolism, excretion and toxicity (ADMET). Metabolism of drugs is influenced by cytochrome P450 enzymes, such as CYP3A4 and CYP2D6. Drug interaction with CYP450 enzymes could pose as potential inhibitor or an inducer of the drug with actions of decreasing or nullifying or even amplifying the enzyme activity. No drug to drug interference was present when SD was co-administered with chemotherapeutic agents CAM and PTX. Passive diffusion in absorption and elimination was associated with almost all of the SD compounds. Particularly, SD chromone C manifested lingering absorption and elimination in AUC [13], a striking characteristic which highlighted naturally derived C from SD, engendered a slow onset of bioactivity and prolonged pharmacological effects. This hallmark is synonymous to properties of Chinese herbal medicine.

Conclusion

In summary, the compounds of the crude plant SD have exhibited a wide diversity of pharmacological effects. SD pharmacological efficacies are rooted in its diverse chromones, coumarins and derivatives. These biochemical structures of SD could provide a strong scaffold for potential drug development for the treatment of various diseases. It should be noted that this review is by no means exhaustive. Further clinical trials would cast further insights into the mechanisms of the chemical constituents of SD and warrant its use as a drug for various diseases.

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Cite this article as: Jenny Kreiner, Edwin Pang, George Binh Lenon, Angela Wei Hong Yang. *Saposhnikoviae divaricata*: a phytochemical, pharmacological and pharmacokinetic review [J]. *Chin J Nat Med*, 2017, **15**(4): 255-264.