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Molecular Mechanism of Inflammatory Signaling and Predominant Role of *Saposhnikovia divaricata* as Anti-inflammatory Potential†

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Abstract – Natural products have always been a pivotal source of new drug development. Dry roots of *Saposhnikovia divaricata* (Turcz.) Schischk. (Umbelliferae) is a perennial herb and is also known as Bang Pung in traditional medicine. Numerous *in vitro* and *in vivo* studies have revealed the diverse pharmacological effects of *S. divaricata* and its role in the treatment of various diseases. This herb has exhibited significant inhibitory effects against inflammation and associated disorders. The present study explored the ethnopharmacological applications and molecular mechanisms behind the anti-inflammatory effects of *S. divaricata* herb and a single compound blockade of multi-signaling inflammatory cascades. Taken together, this review provides insight into the potential role of *S. divaricata* against various inflammatory diseases.
Keywords – *Saposhnikovia divaricata,* Anti-inflammatory, Natural product, Ethnopharmacology, Molecular mechanism.

Introduction

Inflammation is a complex defense mechanism of the body that responds to endogenous or exogenous stimuli (Dinarello, 2010). From a very early stage in phylogenesis, inflammation is associated with the host defense against infectious agents or injury. A variety of exogenous and endogenous mediators are capable of inducing inflammatory responses, such as microbial agents, toxins and pH changes. Inflammation alters tissue homeostasis, immune-cell activation and migration, and it induces the secretion of pro-inflammatory cytokines and mediators in a spatio-temporally coordinated manner (Hotamisligil, 2006). Studies of the molecular mechanisms of inflammatory signaling have identified various mediators (enzymes, and cytokines) and protein kinases that are fundamental signaling components. These components are potential therapeutic targets (Gaestel *et al*., 2009). Inflammation is the principal response of the body to injuries, and the hallmarks of inflammation are swelling, redness, pain and fever (Wellen and Hotamisligil, 2005). This often short-term adaptive inflammatory response is an essential component of tissue repair and involves the integration of several complex signals in distinct cells and organs.

Acute inflammation is typically suppressed after the exclusion of pathogens and cellular debris (Gilroy *et al*., 2004). However, in various diseases such as rheumatoid arthritis, inflammatory bowel disease and bronchial asthma a chronic inflammatory state is maintained, leading to damaging local and systemic effects on host cells and tissues. Inflammation defects are often observed in atherosclerosis, Alzheimer's disease, ischemic heart and brain diseases, and cancer (Dinarello, 2010). The long term consequences of prolonged inflammation are detrimental.

Chronic inflammation is treated by a variety of approaches (Dinarello, 2010). Traditional and conventional treatments include fast-acting symptomatic agents (non-steroidal anti-inflammatory drugs (NSAIDs), glucocorti-coids), slow-acting disease-modifying anti-rheumatic drugs (low-dose methotrexate), and cytostatic agents (azathioprine, cyclophosphamide and high-dose methotrexate) (Dinarello, 2010). This review highlights our under-standing of inflammatory signaling via major signaling pathways, and the active components of S. divaricata are summarized.

Major signaling pathways involved in the inflammatory process

There are several fully established inflammatory signaling

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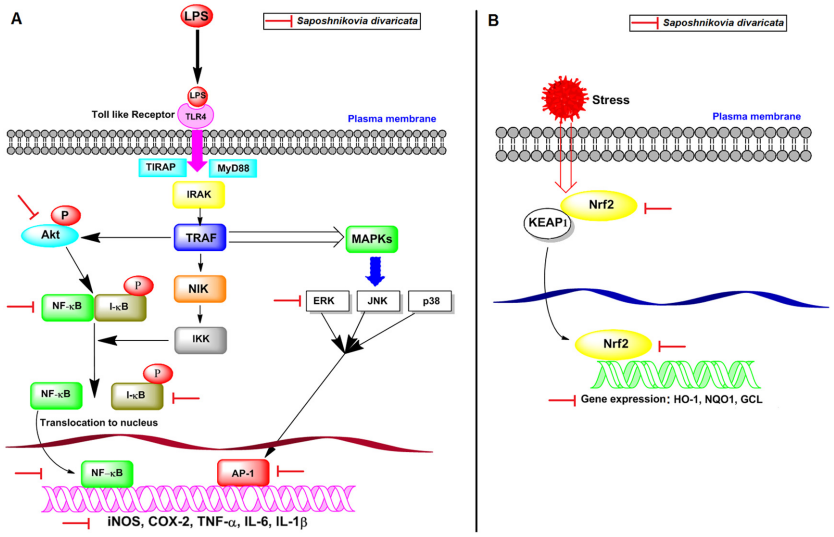

Fig. 1. (A) Molecular signaling pathways involved in inflammatory process and inhibition of *Saposhnikovia divaricata*. (B) Inhibition of signaling pathway involved in oxidative stress and Nrf2 by *Saposhnikovia divaricata*.

pathways. These pathways are highly complex, with many components and layers of post-translational modifications, but rely on only a few major principles of signal transduction. Numerous signaling mechanisms and cells types are involved in the inflammatory response, which presents many potential anti-inflammatory drug targets (Fig. 1) (Nathan, 2002).

NF-κB and inflammation – The most important mechanism by which LPS is sensed is through the LPS-binding protein (LBP)-LPS complex, which then signals through the Toll-like receptor 4 (TLR4)-MD-2 complex (Aderem and Ulevitch, 2000; Shimazu *et al*., 1999). LPS signaling is also activated by cell surface molecules, such as the macrophage scavenger receptor (MSR) (Nathan, 2002). Intracellular signaling depends on the binding of the intracellular TLR domain, TIR (Toll/IL-1 receptor homology domain), to IRAK (IL-1 receptor-associated kinase), a process that is facilitated by two adapter proteins, MyD88 (myeloid differentiation protein 88) and TIRAP (TIR domain containing adapter protein, also called MyD88-adapter-like protein or Mal) and is inhibited by a third protein Tollip (Toll-interacting protein) (Fig. 1).

The MyD88-independent pathway is another pathway by which TIRAP signals through an RNA-dependent protein kinase (PKR) and interferon regulatory factor (IRF)-3. Recently, it has been proposed that cells may also be able to respond to LPS by intracellular receptors called NOD proteins (nucleotide-binding oligomerization domain) (Inohara *et al*., 2001). The NOD proteins are similar to the resistance genes in plants that are involved in pathogen recognition with TLRs (Inohara *et al*., 2002). Expression of NOD1 and NOD2 are responsive to Gram-negative LPS but not to lipoteichoic acid, which is found in Gram-positive bacteria (Hampe *et al*., 2002).

Previous reports have demonstrated that LPS-induced NF-κB pathways can be divided into two distinct signaling pathways known as the canonical and the non-canonical or alternate pathway (Tergaonkar, 2006). The common regulatory step in both of these cascades is the activation of an IκB kinase (IKK) complex consisting of catalytic kinase subunits (IKKα and/or IKKβ) and the regulatory non-enzymatic scaffold protein NEMO (NF-κB essential modulator also known as IKKγ). NF-κB dimers are activated by IKK-mediated phosphorylation-induced proteasomal degradation of the IκB inhibitor. This enables the active NF-κB transcription factor subunits to translocate to the nucleus and induce target gene expression. In the canonical signaling pathway, binding of a ligand to a cell surface receptor, such as a member of the Toll-like receptor superfamily, leads to the recruitment of adaptors (such as TRAF) to the cytoplasmic domain of the receptor. These adaptors then recruit the IKK complex, which leads to phosphorylation and degradation of the IκB inhibitor. The canonical pathway activates NF-κB dimmers comprising RelA, c-Rel, RelB and p50. The alternate or non-canonical pathway utilizes an IKK complex that comprises two IKKα subunits but not NEMO. In the non-canonical pathway, ligand induced activation results in the activation of NF-κB-inducing kinase (NIK), which phosphorylates and activates the

IKKα complex. The IKKα complex then phosphorylates p100, leading to the processing and liberation of the p52/RelB active heterodimer (Hayden and Ghosh, 2004).

Akt/PI3K and inflammatory signaling – Akt/protein kinase B (PKB) is a serine-threonine kinase that has a well-known ability to inhibit cell death and apoptosis signaling pathways (Brazil and Hemmings, 2001; Thomas *et al*., 2002). The activation of Akt is induced by various growth factors and cytokines through the phosphoinositide 3-kinase (PI3K) pathway. Upon stimulation, PI3K phosphorylates specific phosphoinositide lipids, which accumulate in the plasma membrane and create docking sites for Akt. At the plasma membrane Akt is phosphorylated and activated (Fig. 1).

Initially, NF-κB and Akt were thought to be the components of divergent signaling pathways. However, several reports have confirmed the convergence of the NF-κB and Akt signaling pathways (Thomas *et al*., 2002). Indeed, IκB kinase is a substrate of Akt that is also involved in NF-κB activation. Thus, the activation of Akt stimulates NFκB activity.

Mitogen activated protein kinase (MAPK) and inflammatory signaling – The mitogen-activated protein kinases (MAPKs) are a group of signaling pathways that are vital to the regulation of cell differentiation and growth, and the control of cellular responses to cytokines and stresses (Hattori *et al*., 2003). MAPKs are also important in the inflammatory processes. At least three MAPK cascades are well-described: extracellular signal-regulated kinase (ERK), p38, and Jun N-terminal kinase (JNK)/stress-activated protein kinase (Hattori *et al*., 2003). The phosphorylation of MAPKs is known to be a critical component in the production of NO and pro-inflammatory cytokines in activated macrophages (Hattori *et al*., 2003).

Nrf2, oxidative stress and inflammatory signaling – The TLR4 ligand activates several kinases, transcription factors, and the subsequent gene products (Covert *et al*., 2005). LPS can be post-translationally modified by ROS. This has been well recognized in the context of NF-κB translocation to the nucleus and the activation of activator protein 1 (AP-1) transcription factors that regulate gene expression after the LPS stimulation of macrophages (Covert *et al*., 2005). Due to the various sources of ROS within the cell, it has been difficult to identify the critical regulatory factors that control oxidant production at the subcellular level (Fig. 1).

Moreover, the molecular targets of ROS are extensive and pleiotropic and include pro-inflammatory actions, such as cytokines induction, and the simultaneous activation of anti-inflammatory molecules, such as IL-10 and soluble TNF receptors (Zhang *et al*., 2001). Previous reports have illustrated that Nrf2, which encodes a basic region-leucine zipper transcription factor, is a key regulator of the cellular redox state, in part by regulating levels of the intracellular antioxidant glutathione (Thimmulappa *et al*., 2006). Nrf2 activity is regulated by Kelch-like, erythroid cell-derived protein with cap'n'collar homology-associated protein 1 (Keap1), but upon cellular activation, such as oxidative stress or MAPK activation, Nrf2 dissociates, translocates to the nucleus, and binds to cis-acting antioxidant response elements (ARE), which regulate the expression of antioxidant and detoxification genes (Cao *et al*., 2005).

Natural products have been the source of most of the active ingredients in medicines. According to the previous reports, more than 80% of drugs originated from natural products (Harvey, 2008). Likewise, the dry root of *Saposhnikovia divaricata* (Turcz.) Schischk. (Umbelliferae) is a perennial herb that is also known as Bang Pung in traditional herbal medicine. *S. divaricata* possesses analgesic, antipyretic, and antibacterial properties (Khan *et al*., 2011; Kuo *et al*., 2002). The diverse pharmacological applications and the traditional usage of Bang Pung convinced us to study Bang Pung in detail. In this review, ample evidence suggests that Bang Pung and its major components mediate multiple molecular signaling pathways and should be considered to be of therapeutic value for the treatment and prevention of inflammation and inflammatory associated disorders.

Ethnopharmacology and the traditional applications of *S. divaricata*

S. divaricata has a pungent, sweet taste and a warming ability. This herb has effects on the urinary bladder, liver and spleen. Additionally, *S. divaricata* has been used to treat the following disorders:

- Exterior syndrome: wind-heat, wind-damp, Wind-cold, and German measles. Each of these diseases can be treated with *S. divaricata* in combination with other herbs as a formula (Bensky *et al*., 2004).
- Tetanus, spasm, and convulsion: This is due to internal wind, which is briefly defined as the liver heat rising to produce wind. Liver heat can be in the form of excess liver fire, or liver Yin deficiency leading to dominance of liver Yang. The nature of wind is fast and unpredictable, similar to the characteristics of tetanus, spasm, and convulsion. *S. divaricata* is used to effectively mitigate spasms and

treat tetanus (Bensky *et al*., 2004).

- Diarrhea due to the liver overacting on the spleen: This is also known as Irritable Bowel Syndrome (IBS) (Malagelada, 2006). Excess liver heat and overactivity of the spleen can cause diarrhea, known as IBS.

Treatment of migraines and headaches caused by the common cold by *S. divaricata* – Migraines are a chronic neurological disorders characterized by severe headaches on one side of the head (Bartleson and Cutrer, 2010). The traditional uses of *S. divaricata* include a decoction of nine grams of *S. divaricata* herb and six grams of Angelica Dahuricae Radix (Baek Ji) was treating migraine pain and plus Chuan Xion Chuanxiong Rhizoma (Chuan Xion) and Schizonepetae Spica (Hyeong Gae) for the treatment of headache in common cold (Yan *et al*., 1977).

Treatment of Bi syndrome by *S. divaricata* – In traditional medicine, Bi syndrome is the pain sensation due to the disruption in or lack of Qi flow. *S. divaricata* is commonly used in traditional medicine clinics to treat this illness (Yan *et al*., 1977).

Treatment of Rosacea by *S. divaricata* – Rosacea is a chronic condition characterized by facial redness and pimples (Wollina and Verma, 2009). The traditional treatment of rosacea is a mixture containing *S. divaricata* as the principle ingredient and eleven other herbs (Yan *et al*., 1977).

Other species of Bang Pung

Peucidenum japonicum: P. japonicum is a perennial herb that belongs to the Umbelliferae family. It has been reported that several compounds isolated from this roots of this plant have pharmacological activities (Hata *et al*., 1968; Hsiao *et al*., 1998). The root (Peucedani Radix) was used to treat coughs, headaches and pain in Korean folk medicine (Bensky *et al*., 2004).

Glehnia littoralis: Glehnia littoralis (Umbelliferae) is a perennial herb used as remedy for cough (Li *et al*., 2008), and some other traditional oriental medicines have used this herb as a diaphoretic, antipyretic, and analgesic agent (Yoon *et al*., 2010).

Phytochemistry and the major components of *S. divaricata*

An exhaustive literature survey of the phytochemical reports of the genus *S. divaricata* revealed that the main root of *S. divaricata* contained 0.09~0.11% essential oil. The major components of *S. divaricata* are octanal, β-bisabplene, nonanal, 5-O-methylvisamminol, saposhniukovan A, saposhnikovan C, saposhnikovan B, β-sitosterol, daucosterol, mannitol, sucrose, lignoceric acid, deltoin, xanthotoxin, anomalin, imperatorin, sec-glucosyhamaudol, hamaudol, cimifugin, prim-O-glucosyl-cimifugin, nodakentin, 2-butene diacid, 4-hydroxy-3-methoxy-benzoid acid, adenosine, ledebouliellol, bergapten, fraxitin, scopoletin, psoralen and marmeinen. Fig. 2 represents the chemical structures of the most commonly occurring major compounds from *S. divaricata*.

Mechanism and anti-inflammatory role of *S. divaricata* in inflammation and associated diseases

Anti-inflammatory properties of *S. divaricata* – Many reports have demonstrated that *S. divaricata* and several components of *S. divaricata* exhibited significant anti-inflammatory properties. The anti-inflammatory activity of *S. divaricata* is associated with signaling pathways. The EtOH extract of *S. divaricata* showed a remarkable suppression of iNOS and COX-2 expression levels at nontoxic concentrations in LPS-stimulated macrophages (Tai and Cheung, 2007). According to previous studies, imperatorin and deltoin, the major components of *Saposhnikoviae* Radix, suppress NF-κB and MAPK activation in RAW 264.7 macrophages (Guo *et al*., 2012; Wang *et al*., 1999). Khan *et al*. illustrated that anomalin, a pyranocoumarin derivative that is isolated from *S. divaricata*, inhibited inflammatory signaling through various inflammatory signaling cascades. Molecular analysis revealed that several pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), were reduced by anomalin, and this reduction correlated with the down-regulation of the NF-κB signaling pathway (Khan *et al*., 2011).

Anti-nociceptive and analgesic properties *S. divaricata* – Previous studies have shown that *S. divaricata* exhibited a strong inhibitory effect against various inflammatory models. Because pain is a classic symptom of inflammation, pain signaling is closely related to inflammatory pathways. *Saposhnikovia* root showed an analgesic and anti-nociceptive effect in an acetic acid-induced mouse model (Okuyama *et al*., 2001). Similarly, single compounds, such as chromones (divaricatol, ledebouriellol and hamaudol), isolated from *S. divar*icata exhibited a potent analgesic effect in mice. Acylglycerols and sec-O-glucosylhamaudol were also shown significantly inhibit pain (Okuyama *et al*., 2001).

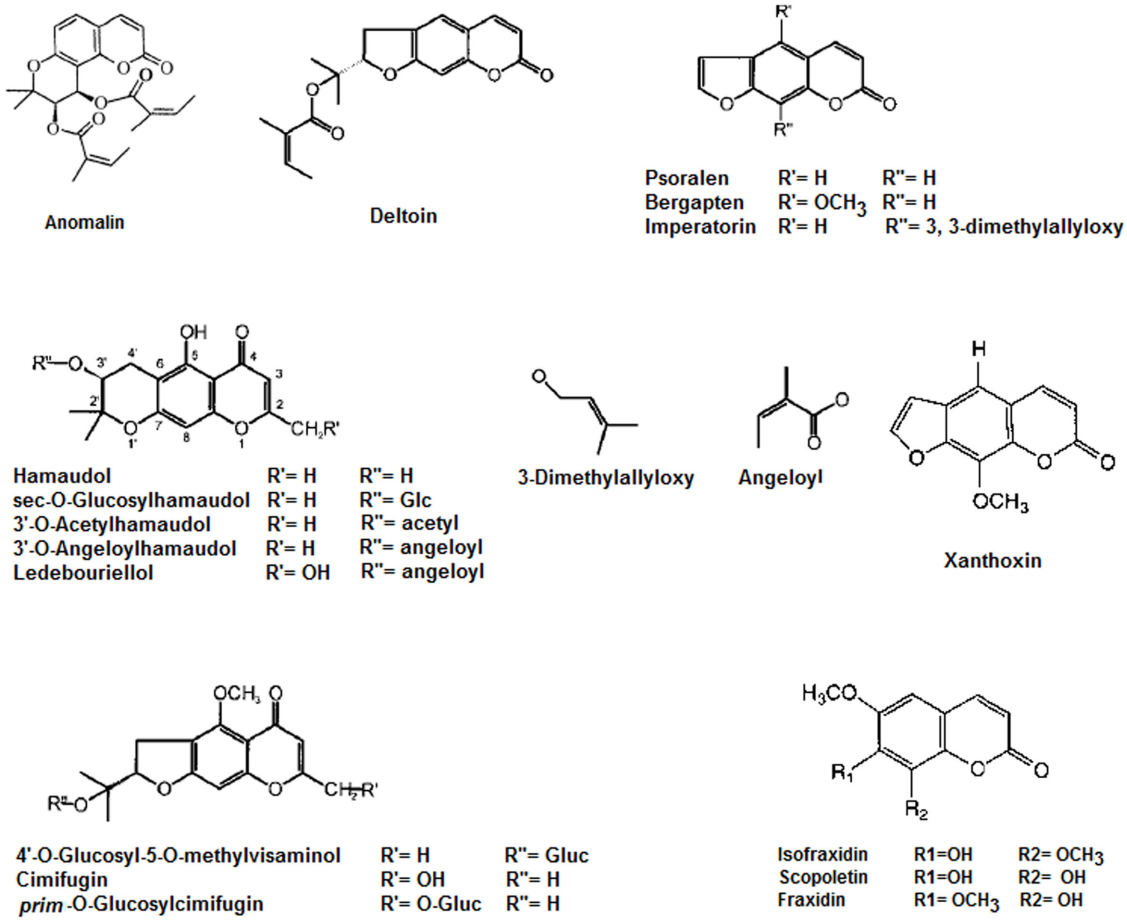

Fig. 2 Chemical structures of isolated compounds from *S. divaricata* roots.

Nerve protective and LPS-induced lung protection properties of *S. divaricata* – Peripheral nerve injury an important and frequent injury (Dinh *et al*., 2009). The release of pro-inflammatory mediators and ROS at the site of an injury modulate the inflammatory response and are involved in necrosis and apoptosis after nerve injury (Dinh *et al*., 2009). The search for effective drugs to promote nerve regeneration has become a therapeutic need. Recently, various reports have shown that traditional medicines are effective in the treatment of peripheral nerve injury and nerve regeneration. *S. divaricata* has been reported to have nerve protective properties in combination with other herbs (Kim *et al*., 2011). *S. divaricata* exhibited remarkable protection effects against nerve and lung injury through the suppression of pro-inflammatory cytokines and the NF-κB pathway (Ge *et al*., 2007).

Anti-oxidant properties of *S. divaricata* – Anti-oxidants are molecules that inhibit the oxidation of other molecules. Oxidative stress is an important part of numerous diseases, and the applications of anti-oxidants, particularly as treatments for stroke and neurodegenerative diseases, are of great interest. Additionally, oxidative stress is both the rationale and the result of a disease state (Sies, 1997). *S. divaricata* possesses significant anti-oxidant activity alone or in combination with other herbs. Polysaccharides isolated from *S. divaricata* had some functions of scavenging free radicals and inhibiting lipid peroxidation, and the scavenging effects on DPPH and hydroxyl radicals (OH) were especially significant (Tai and Cheung, 2007; Zhang *et al*., 2008). Bo Zhao *et al*. demonstrated that a single compound (cimifugin and 5-O-methylvisamminol) isolated from *S. divaricata* showed significant anti-oxidant activity (Zhao *et al*., 2012).

Anti-convulsive properties of *S. divaricata* – Inflammatory signaling contributes to the pathogenesis of numerous neurodegenerative disorders, including Alzheimer's disease, neurological infection, ischemic stroke, and traumatic brain injury (Glass *et al*., 2010). Inflammation is a natural physiological response that is mediated by the

Fig. 3 Pharmacological applications of *S. divaricata.*

innate immune system. Increasing evidence supports a connection between inflammation and epilepsy, both in terms of epileptogenesis and the long-term consequences of seizures (Vezzani *et al*., 2011). Recent reports constitute the basis of a novel approach to developing an epilepsy treatment that is distinct from previous approaches and is uniquely based on sound neurobiological evidence. Wang *et al*. reported that the water extract of *S. divaricata* showed significant anti-convulsive effects (Wang *et al*., 1991). A study performed previously demonstrated that an intragastric dose of *S. divaricata* extract exhibited a 60% reduction in electric shocks in albino mice (Yan *et al*., 1977).

Anti-proliferative and anti-tumor properties of *S. divaricata* – The connection between inflammation and cancer has been established in recent years, based on observations that tumors often arise at sites of chronic inflammation and inflammatory cells (Mantovani *et al*., 2008). The hallmarks of cancer that are related inflammation include the presence of inflammatory cells and inflammatory mediators (for example, chemokines, cytokines and prostaglandins) in tumor tissues, tissue remodeling and angiogenesis similar to that seen in chronic inflammatory responses, and tissue repair (Coussens and Werb, 2002). Kuo *et al*. illustrated that *S. divaricata* ethanol extract potently inhibited the proliferation of various tumor cells (Kuo *et al*., 2002). *S. divaricata* ethanol extract possesses strong anti-proliferative properties against several human tumor cell lines, and a mild granulocyte differentiation- inducing property in HL60 cells (Tai and Cheung, 2007).

Conclusion

This review summarized the speculated molecular mechanisms involved in inflammatory signaling and inhibitory role of *S. divaricata* (Fig. 1 and Fig. 3). It has been clearly demonstrated that *S. divaricata* modulates various molecular targets and exerts versatile pharmacological and biological properties, including anti-inflammatory activity for the treatment and prevention of acute and chronic inflammatory disorders. Several animal and cellular models explored the diverse pharmacological applications of the *S. divaricata* herb. *S. divaricata* exhibited significant inhibitory effects against inflammation and associated disorders. Various single components from *S. divaricata* act as inflammatory therapeutic agents, which are increasingly needed to combat select pathologies. More in depth investigations are required to provide further insight into the signaling that are mediated by *S. divaricata* and isolated compounds, which have efficacious therapeutic applications.

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