


# Rationale of *Orthosiphon aristatus* for Healing Diabetic Foot Ulcer

Natural Product Communications  
Volume 15(9): 1–13  
© The Author(s) 2020  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1934578X20953308  
journals.sagepub.com/home/npx



Farah Izana Abdullah<sup>1,2</sup>, Lee Suan Chua<sup>1,2</sup> ,  
Siti Pauliena Mohd Bohari<sup>3</sup>, and Eka Sari<sup>4</sup>

## Abstract

*Orthosiphon aristatus* (Blume) Miq. is traditionally used for wound healing in South East Asia and scientifically proven for its antidiabetic potential. Wounds due to diabetes, especially diabetic foot ulcer (DFU), always involve a complicated healing process. The present work aims to review the information on the rationale of the phytochemicals from *O. aristatus* in promoting DFU healing. The findings showed that the DFU healing potential of *O. aristatus* was characterized by a reduction in the blood glucose level, mainly attributed to the significant concentration of constituents such as caffeic acid, rosmarinic acid, and sinensetin in the plant extract. These phytochemicals possibly induce insulin secretion and sensitivity, improve the lipid profile, and stimulate glucose uptake. Furthermore, the healing effect may also be contributed to the antioxidant, anti-inflammatory, and antihyperglycemic properties of the plant. The roles of phytochemicals have been systematically postulated in the 4 phases of the healing process. Moreover, no adverse toxic sign or abnormality has been reported upon oral administration of the plant extract. This suggests that *O. aristatus* extract could be a potential diabetic wound healing phytomedicine for further preclinical and clinical studies.

## Keywords

*Orthosiphon aristatus*, phytochemicals, bioactivity, wound healing, antidiabetic potential

Received: June 10th, 2020; Accepted: August 4th, 2020.

## Introduction

About 10% of 250 000 plant species have been scientifically studied and found to have potential uses in healthcare.<sup>1,2</sup> Some of the plant-derived compounds such as vinblastine, vincristine, taxol, and digoxin have been synthesized because of the high demand and limited supply from natural resources.<sup>3,4</sup> Recent development of traditional medicines has led to the extensive use of natural products and their derivatives which contribute to more than half of the total medicines consumed worldwide.<sup>5</sup>

One of the widely used medicinal plants in Asia is *Orthosiphon aristatus* (Blume) Miq. This herb, belonging to the family Lamiaceae, is a traditional medicinal plant originating from tropical East Asian countries such as Thailand, Indonesia, Vietnam, and Malaysia.<sup>6</sup> The leaves of the herb are popularly consumed as tea to promote overall well-being. A decoction of the herb is traditionally prepared as a remedy for arteriosclerosis, kidney stones, diabetes, and nephritis.<sup>7</sup> The herb is also extensively used as an ethnomedicine to treat rheumatism, hypertension, tonsillitis, epilepsy, menstrual disorder, gonorrhoea, syphilis, renal calculus, gallstone, lithiasis, edema, eruptive fever, influenza, hepatitis, and jaundice.<sup>8-12</sup>

Many pharmacological studies have demonstrated that this herb exhibits antimicrobial, antioxidant, hepatoprotective, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, antidiabetic, and anti-inflammatory activities.<sup>13</sup> The pharmacological properties are most probably attributable to the presence of various groups of phenolic acids,<sup>14-19</sup> terpenoids (diterpenes and triterpenes),<sup>7,20-22</sup> flavonoids,<sup>14,15</sup> and benzochromenes.<sup>23</sup> Phenolic compounds, such as caffeic acid, rosmarinic acid, sinensetin, and eupatorin, have been frequently reported to be chemically bioactive constituents of *O. aristatus*.<sup>7,24-27</sup> These compounds are likely

<sup>1</sup>Institute of Bioproduct Development, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia

<sup>2</sup>Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Johor Bahru, Johor, Malaysia

<sup>3</sup>Department of Bioscience, Faculty of Science, Universiti Teknologi Malaysia, Johor, Malaysia

<sup>4</sup>Bioengineering and Biomedical Engineering Laboratory, Research Centre of Sultan Ageng Tirtayasa University, Serang, Banten, Indonesia

## Corresponding Author:

Lee Suan Chua, Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor Bahru, Johor, Malaysia.  
Email: chualeesuan@utm.my



responsible for the wound contraction and enhancement of the rate of epithelialization.<sup>28-33</sup> Moreover, *O. aristatus* is popularly known as an alternative antidiabetic medicine, specifically for type II diabetes.<sup>34</sup> Many in vitro and in vivo studies have also been conducted to evaluate the antidiabetic activity and toxicity of *O. aristatus*.<sup>35-39</sup> Hence, this current review aims to rationalize the potential of *O. aristatus* as a phytomedicine for the healing of diabetic foot ulcer (DFU), mainly focusing on the role of bioactive phytochemicals, their mechanisms of action, and toxicity studies on diabetic-induced animal models.

An intensive literature survey was conducted using the following databases: PubMed, Google Scholar, Scopus, and Science Direct. The keywords used for the search were “*Orthosiphon aristatus*,” its synonym “*Orthosiphon stamineus*,” “phytochemical,” “caffeic acid,” “rosmarinic acid,” “sinensetin,” “antimicrobial,” “antidiabetic” and “diabetic foot ulcer healing.” No limit was set for the time frame of published articles in order to retrieve all relevant papers, and the last search was performed on February 21, 2020.

## Wound Formation and Healing

Skin is the body’s largest organ and plays a crucial role as a protective barrier against environmental insult.<sup>40</sup> Wounds are a type of injury that results in either the opening or breaking in the epidermis layer of skin.<sup>41,42</sup> An acute wound is a tissue injury for which the reparative process follows an orderly process to sustain restoration and functional integrity. Usually, an acute wound can be healed within 3 weeks, starting from the initial insult.<sup>40,43</sup> Possibly, an acute wound can progress into a chronic wound when the healing process is delayed by up to 12 weeks. This will result in the interruption of all skin layers and a delay in closing the wound gap. A chronic wound does not follow the normal stages of healing and requires a prolonged time to heal or it will recur frequently.<sup>44-46</sup> Chronic wounds usually happen due to the complication of some diseases such as diabetes, spinal cord injuries, and Pick’s disease.<sup>40,47</sup>

Wound healing is a natural body reaction in response to an injury involving biochemical and physiological phenomena that behave in a synergistic manner. Wound healing occurs in 4 stages. The 4 overlapping district phases of healthy wound healing can be categorized as homeostasis, inflammation, proliferation, and remodeling.<sup>48,49</sup> The first phase is homeostasis, which controls excessive blood loss from the damaged vessels. During the homeostasis stage, the body will release chemical mediators and intercellular messengers (growth factors) to begin the wound cleaning and healing process.

The second phase of the wound healing process is inflammation and debridement. After stopping blood loss (coagulating), the body will immediately send plasma proteins, blood cells, and antibodies to the wound area as a defense mechanism

at the inflammatory phase. This causes swelling, pain, fever, and redness around the wound site, and the symptoms could last for up to 2-4 days.<sup>46,48,50</sup>

The proliferation stage starts when the inflammation subsides. Dermal fibroblasts migrate to the wound site and start granulating until the wound is healed.<sup>46,48,50</sup> The scab sloughs off when the epidermis has been restored to standard thickness during the remodeling or maturation phase. Therefore, the third phase is re-epithelialization, which includes proliferation, migration, and differentiation of squamous epithelial cells in the epidermis. The final stage of wound healing involves collagen deposition and remodeling within the dermis layer of skin.<sup>51</sup> Ultimately, collagen fibers become more organized, fibroblasts decrease in number, and blood vessels are restored to normal.<sup>46,48,50</sup> Understanding the process of wound formation and healing would be of importance in the search for natural alternatives for addressing this complication.

## Complications of Diabetic Foot Ulcer

Diabetic patients are facing a complicated wound healing cascade. The healing process is delayed and disrupted from following the normal wound-healing process.<sup>44,52</sup> The healing process of diabetic wounds is relatively slow. Previous studies explained that a high glucose concentration was the main factor inhibiting the wound-healing process and was always associated with a prolonged inflammatory phase.<sup>46</sup> This is because the cell walls become rigid, which causes difficulty for blood flow through small vessels at the wound surface. This also obstructs the permeability and flow of red blood cells. Such a condition will deteriorate oxygen release and nutrient deficit at the wound site.<sup>53</sup> When this happens, blood glucose will be elevated, and chemotaxis and phagocytosis are being agonized to control the infection in the wound area. Consequently, this delays macrophage introduction and diminishes leukocyte migration, as well as prolonging the inflammatory stage in the wound healing cascade.<sup>53</sup>

DFU is characterized by the presence of a full-thickness, prolonged wound below the ankle of people with diabetes, or a lesion of the foot penetrating through the dermis layer.<sup>54</sup> DFU does not follow the orderly process of wound healing. It is always associated with poor glycemic (blood glucose) control. A study showed that 49% of participants who had foot ulcers had a glycated hemoglobin (glycemic measure) level above 8.4%.<sup>55</sup> Thus, chronic hyperglycemia appears to be one of the most important factors in the development and delayed healing of DFUs.<sup>56-59</sup>

Hyperglycemia results in the activation of the polyol pathway, nonenzymatic glycosylation, and formation of advanced glycation end products (AGEs), diacylglycerol-(DAG) protein kinase C pathway, and overactivity of the hexosamine pathway.<sup>60,61</sup> All 4 mechanistic pathways will lead to mitochondrial overproduction of reactive oxygen species (ROS).<sup>62</sup> ROS are known to promote cellular dysfunction, thus leading to damage of deoxyribonucleic acid synthesis, lipid and amino acid

oxidation, and enzyme inactivation involved in metabolic function. Moreover, hyperglycemia also leads to the activation of an inflammatory response via the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B).<sup>63-65</sup> All these factors delay the healing of foot ulcers. *Orthosiphon aristatus* and related plant species in the same family have been scientifically proven to lower blood glucose level<sup>66,67</sup> and also possess antioxidant<sup>32,68</sup> and anti-inflammatory<sup>69-71</sup> activities. Therefore, *O. aristatus* and its phytochemicals can be a potential lead in healing DFU.

## Low Glycemic Index Helps Diabetic Foot Ulcer

It is known that the glycemic measure is frequently poor in people with diabetes, and, therefore, normoglycemia is important for managing foot ulcer, in the belief that it will enhance healing.<sup>72</sup> The adverse effects on cellular immunity and infection can be reduced by controlling the blood glucose level.<sup>73</sup>

Several observational studies found a positive correlation of glycemic control and wound healing.<sup>74-76</sup> Type 2 diabetic patients with proper glycemic control could have a 35% reduction of amputation risk in the lower extremity of the body.<sup>77</sup> Intensive glycemic control also leads to a reduction in the progression and development of microvascular (small vessel) complications, including diabetic peripheral neuropathy.<sup>78</sup>

The wound morphology and proliferation of fibroblasts is abnormal for people with DFU. There is also a glucose-dependent reduction of keratinocyte proliferation and differentiation.<sup>79,80</sup> Both insulin and insulin-like growth factor-1 were observed to have a beneficial effect on wound healing in experimental animals.<sup>81,82</sup> Therefore, a low glycemic index is strongly recommended for wound healing. This is because hyperglycemia, insulin resistance, dyslipidemia, and oxidative stress play a dominant function in the pathogenesis of DFU.<sup>73,83,84</sup>

## Herbs With Diabetic Foot Ulcer Healing Potential

The wound healing potential of a plant extract may be due to the presence of bioactive phytochemicals which have been reported to improve its repair mechanism. Many medicinal plant species synthesize equivalent or closely related compounds with similar biological properties and share the same biological targets and pathways. For example, acemannan from *Aloe vera*, hydroxysafflor yellow A from *Carthamus tinctorius*, polysaccharides from *Ganoderma lucidum* and *Sanguisorba officinalis*, phthalide lactones and alkaloids from *Ligusticum striatum*, saponins from *Panax ginseng*, shikonin and arnebin-1 from *Lithospermum erythrorhizon*, salvianolic acid from *Salvia miltiorrhiza*, and alkaloid and stilbenoid from *Stemona tuberosa* have been well characterized and demonstrated to exhibit the properties of wound healing. Salvianolic acid was also detected in *O. aristatus* in the study of Nuengchamngong et al.<sup>17</sup> The

compounds mostly target mitogenic pathways (eg, phosphokinase B, phosphatidylinositol-3-kinase, SMAD, and cyclins), proinflammatory NF- $\kappa$ B pathways (eg, caspases, interleukins, tumor necrosis factor- $\alpha$ , and tumor growth factor- $\beta$ 1), angiogenesis pathways (eg, vascular endothelial growth factor), extracellular matrix synthesis (eg, matrix metalloproteinases), and differentiation pathways (eg,  $\alpha$ -smooth muscle actin), which are the key routes in the mammalian wound healing cascade.<sup>5</sup>

As suggested by the Chinese Pharmacopeia,<sup>85</sup> the combination of *Cornus officinalis* (dried ripe sarcocarp of Fructus Corni), *Schisandra chinensis* (dried ripe fruit of Fructus Schisandrae Chinensis), *Poria cocos* (dried sclerotium of Poria), *Alisma orientalis* (dried tuber of Rhizoma alismatis), and *Dioscorea opposita* (dried rhizome of Rhizoma dioscoreae) are traditionally used for diabetic treatment, either as a single herb or a cluster of herbs in a traditional formula (Liuwei Dihuang Wan) for diabetes treatment.<sup>86</sup> They are also commonly used in traditional Chinese medicine for the treatment of antidiabetic foot ulcer with proven clinical efficacy. The effectiveness was associated with the normalization of glycemic control in diabetes.<sup>87</sup> A simple herbal formula consisting of *Astragalus* spp. (*Astragali Radix*) and *Rehmannia glutinosa* (*Rehmanniae Radix*) could expedite the healing of DFUs by inducing gene expression implicated in fibroblast regeneration, angiogenesis, and anti-inflammation, thus promoting vascularization and granulation, as well as modulating the inflammatory response.<sup>5</sup>

## Involvement of Phytochemicals in the Healing Mechanism

The majority of phytochemicals found in *O. aristatus* are phenolic acids, flavonoids, and terpenes, as presented in Table 1. The major constituents are rosmarinic acid, eupatorin, and sinensetin.<sup>16,24</sup> Flavonoids (sinensetin) and terpenoids (limonene, borneol, linalool, camphor, and eugenol) are known to promote wound healing due to their antimicrobial properties.<sup>88-91</sup> These compounds have remarkable antioxidant and antiulcer activities.<sup>92</sup> The contribution of phytochemicals in *O. aristatus* in wound healing can be explained in Figure 1. The figure postulates and explains the role of each class of phytochemicals in detail. In the homeostasis phase, triterpenes (oleanolic acid and ursolic acid) help in wound healing by producing and activating inflammatory mediators and growth factors, and thus enhancing wound contraction and the rate of epithelialization.<sup>93-95</sup> Since the inflammatory phase is overlapping with the proliferative phase, the presence of excessive ROS may delay the wound-healing process. Plant-derived antioxidants, such as triterpenes, flavonoids, and phenolics, can be potent radical scavengers, and thereby preventing the damage due to free radicals during the wound-healing process.<sup>33,96</sup> Triterpenes also function to modulate the production of ROS in the wound microenvironment and induce cell migration, cell proliferation, and collagen deposition, thus accelerating the process of tissue repair.<sup>97</sup> Phenolics (chicoric acid, lithospermic acid, and

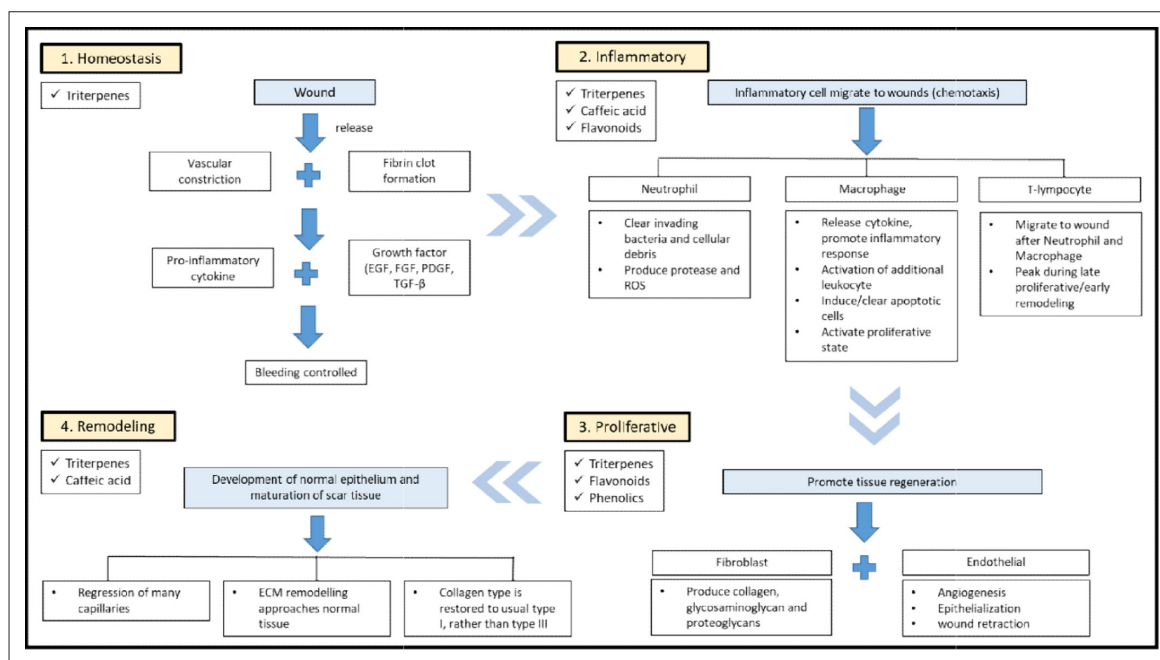
**Table 1.** Phytochemicals of *Orthosiphon aristatus* and Their Reported Bioactivities.

Plant part	Group	Compound	Bioactivities	Reference	
Leaves	Phenolic acid	Rosmarinic acid	Antihyperglycemic, anti-inflammatory, antioxidant, antibacterial	14-18	
		Caffeoyl tartrate	Antioxidant	15	
		Aurantiamide acetate	Anti-inflammatory	15	
		2,3-Dicaffeoyl tartrate	Antioxidant	15	
		Caffeic acid	Antioxidant	15,17	
		Danshensu	Antihyperglycemic, anti-inflammatory, antioxidant	17	
		Sagerinic acid	Antioxidant	17	
		Salvianolic acid B	Antioxidant, anti-inflammatory	17	
		Caftaric acid	Antioxidant, anti-inflammatory	17	
		Lithospermic acid	Antioxidant, anti-inflammatory	19	
		Chicoric acid	Antioxidant, anti-inflammatory	19	
		Triterpene	Ursolic acid	Antioxidant, anti-inflammatory	7,20
			Oleanolic acid	Antioxidant, anti-inflammatory	7,20
			Betulinic acid	Antioxidant, anti-inflammatory	20
			Hydroxybetulinic acid	Antioxidant, anti-inflammatory	20
			$\alpha$ -Amyrin	Antioxidant, anti-inflammatory	20
			$\beta$ -Amyrin	Antioxidant, anti-inflammatory	20
			Maslinic acid	Antioxidant, anti-inflammatory	20
	Flavonoid		3'-Hydroxy-5,6,7,4'-tetramethoxyflavone	Antihyperglycemic, anti-inflammatory, antioxidant	7
		Vomifoliol	Anti-inflammatory, antioxidant	7	
		Sinensetin	Antihyperglycemic, anti-inflammatory, antioxidant	19,21,22,103	
		Eupatorin	Antihyperglycemic, anti-inflammatory, antioxidant	21,103	
		Ladanein	Anti-inflammatory, antioxidant	7	
		7',3',4'-Tri-O-methyluteolin	Antioxidant	7	
		6-Hydroxy-5,7,4'-trimethoxyflavone	Antioxidant	7	
		Tetramethylscutellarein	Antioxidant	21	
		5-Hydroxy-6,7,3',4'-tetramethoxyflavone	Antioxidant	21	
		3'-Hydroxy-5,6,7,4'-tetramethoxyflavone	Antioxidant	21,22,68,103	
	5,6,7,4'-Tetrahydroxyflavone	Antioxidant	19		
	5,6,7,3',4'-Pentahydroxyflavone	Antioxidant	19		
	Pillion	Antioxidant	21		
	Salvegenin	Antioxidant	21		
	Cirsimaritin	Antioxidant	21		
	Rhamnazin	Antioxidant	21		
	Apigenin trimethyl ether	Antioxidant	21		
	Luteolin tetramethyl ether	Antioxidant	21		

(Continued)

**Table 1.** Continued

Plant part	Group	Compound	Bioactivities	Reference		
Aerial part	Diterpene	Orthosiphols A–Z	Anti-inflammatory	15,24		
		Staminols A–D	Anti-inflammatory	15,24		
		Staminolactones A	Anti-inflammatory	15		
		Staminolactones B	Anti-inflammatory	15		
		Norstaminol A	Antioxidant, anti-inflammatory	15		
		Orthosipholl S	Anti-inflammatory	15		
		Secoorthosiphols A–C	Anti-inflammatory	10,23		
		Nororthosiphonolide	Anti-inflammatory	23		
		Norstaminolactone A	Anti-inflammatory	23		
		Norstaminols A–C	Anti-inflammatory	7,23,104,105		
		Norstaminone A	Anti-inflammatory	25		
		Neoorthosiphols A–B	Anti-inflammatory	9,10,106,107		
		Niphonols A–E	Anti-inflammatory	104		
		Orthochromene A	Anti-inflammatory	107		
	Triterpene	Oleanolic acid	Antioxidant, anti-inflammatory	15		
		Ursolic acid	Antioxidant, anti-inflammatory	15		
		Betulinic acid	Antioxidant, anti-inflammatory	15		
		$\beta$ -Sitosterol	Antioxidant, anti-inflammatory	15		
	Flavonoid	7,3,4-Tri- <i>O</i> -methylfluteolin	Antioxidant	15		
		Eupatorin	Antioxidant, antihyperglycemic, anti-inflammatory	14,15		
		Sinensetin	Antioxidant, antihyperglycemic, anti-inflammatory	14,15		
		3'-Hydroxy-5,6,7,4'-tetramethoxyflavone	Antioxidant	15		
		Salvigenin	Antioxidant	15		
		Ladanein	Anti-inflammatory, antioxidant	15		
		Tetramethylscutallarein	Antioxidant	15		
		6-Hydroxy-5,7,4-trimethoxyflavone	Antioxidant	15		
		Kaempferol-3- <i>O</i> - $\beta$ -glucoside	Anti-inflammatory, antioxidant	15		
		Quercetin-3- <i>O</i> - $\beta$ -glucoside	Anti-inflammatory, antioxidant	15		
		Essential oil	Terpene	$\beta$ -Caryophyllene	Antifungal	28
				$\alpha$ -Humulene	Antifungal	28
				$\beta$ -Elemene	Antifungal	28
				1-Octen-3-ol	Antifungal	28
$\beta$ -Bourbonene	Antifungal			28		
$\beta$ -Pinene	Antifungal			28		
Caryophyllene oxide	Antifungal			28		
Camphene	Antifungal			28		
Limonene	Antifungal			28		
$\alpha$ -Pinene	Antifungal			28		
1,8-Cineol	Antifungal			28		
Borneol	Antifungal			28		
Linalool	Antifungal			28		
Camphor	Antifungal			28		
Eugenol	Antifungal	28				
<i>p</i> -Cymene	Antifungal	28				
Barvone	Antifungal	28				
Bornyl acetate	Antifungal	28				
$\delta$ -Badinene	Antifungal	28				
Leaves and flower	Benzochromene	Bethylripariochromene A	Anti-inflammatory	108		



**Figure 1.** The contribution of phytochemicals in *Orthosiphon aristatus* extract in wound healing. In the homeostasis phase, triterpenes in *O. aristatus* are believed to enhance wound contraction and the rate of epithelialization. The inflammatory phase is overlapping with the proliferative phase, and thus, triterpenes, flavonoids, and phenolics can be potent radical scavengers to enhance the wound-healing process by inducing cell migration, cell proliferation, and collagen deposition, enhancing the viability of collagen fibrils to increase the strength of collagen fibers and accelerate the process of tissue repair. In the remodeling phase, caffeic acid helps to increase collagen synthesis in fibroblast cells and control melanin production by inhibiting tyrosinase activity.

rosmarinic acid) and flavonoids (eupatorin) have been shown to significantly reduce tissue lipid peroxidation level.<sup>98-100</sup> This will enhance the viability of collagen fibrils by increasing the strength of collagen fibers, preventing cell damage, and accelerating DNA synthesis.<sup>51</sup> During the remodeling phase, caffeic acid was found to help an increase in collagen synthesis in fibroblast cells and controlling melanin production by inhibiting tyrosinase activity.<sup>101</sup> A group of researchers from Indonesia prepared an *O. aristatus*-based functional drink and reported that it could restrain the increase in blood glucose and inhibit the damaging rate of pancreatic beta cells in diabetic mice.<sup>102</sup> One of the reported bioactive compounds in the functional drink was sinensetin, besides other plant constituents from the polyherbal formulation.

#### Antihyperglycemic Property of Plant Extract

*Orthosiphon aristatus* is popularly known as an antidiabetic alternative medicine for type II diabetes. Many in vitro and in vivo studies have been conducted to evaluate the antidiabetic activity and toxicity of *O. aristatus*, mostly using aqueous or aqueous ethanol extract of the plant leaves. The 50% ethanolic extract of the herb was found to exert in vitro antidiabetic activity by inhibiting  $\alpha$ -glucosidase (half-maximal inhibitory concentration [IC<sub>50</sub>] 4.63 mg/mL) and  $\alpha$ -amylase (IC<sub>50</sub> 36.70 mg/mL).<sup>34,66</sup> Earlier, a 14-day oral treatment was carried out using an aqueous extract of the herb. Investigation was conducted on plasma glucose and lipid profile in normal and

streptozotocin-induced diabetic Wistar rats. The results showed that the administration of *O. aristatus* aqueous extract at 1000 mg/kg exerted hypoglycemic and antihyperglycemic effects. The reduction of plasma glucose levels in both euglycemic and hyperglycemic animals was also observed when the plant extract was administered orally at 200-1000 mg/kg.<sup>13,109</sup> A similar finding was also obtained from the study conducted by Sriplang et al.<sup>110</sup> who used an aqueous extract of *O. aristatus* to alleviate hyperglycemia and improve the lipid profile in diabetic rats. The extract at 1 g/kg was effective in decreasing the plasma glucose concentration, and the response was close to the result of glibenclamide (5 mg/kg). By the end of the study, plasma triglyceride concentration was lower in the extract-treated diabetic rats than untreated rats.<sup>110</sup> Furthermore, plasma high-density lipopolysaccharide-cholesterol concentration was significantly increased in diabetic rats treated with the extract. Moreover, the plant extract (100  $\mu$ g/mL) could stimulate glucose-induced insulin secretion. Therefore, the use of *O. aristatus* extract is beneficial to diabetic patients, especially for those who have a defect in insulinotropic response.<sup>110-112</sup>

*Orthosiphon aristatus* aqueous extract was given orally to streptozotocin-induced Sprague Dawley rats. The experiments were conducted to evaluate the potential of the extract for managing maternal hyperglycemia and to understand the mechanism of actions in lowering blood glucose levels. The extract was effective in lowering the blood glucose level in both nonpregnant and pregnant rats, partly via the stimulation of insulin release which could

probably be triggered by several peptide interactions such as ghrelin and glucagon-like peptide 1. Moreover, no sign of toxicity and mortality was recorded on nonpregnant and pregnant rats throughout the study. This indicated that *O. aristatus* did not induce systematic toxicity.<sup>37</sup> The researchers also suggested that this herb is likely to be a potential antidiabetic agent to treat glucose intolerance during pregnancy. The antidiabetic activity of *O. aristatus* extract was most probably due to the presence of rosmarinic acid and eupatorin. Chemical screening of the extract showed it to have phenolic and flavonoid contents of  $13.24 \pm 0.33$  mg/g and  $1.73 \pm 0.14$  mg/g, respectively.<sup>110</sup> The researchers suggested that sinensetin could be the compound responsible for the glucose reduction with an IC<sub>50</sub> value (50% inhibition) of 0.66 g/mL for  $\alpha$ -glucosidase and 1.13 mg/mL for  $\alpha$ -amylase. They also stated that sinensetin outperformed the reference drug, acarbose.<sup>66</sup> However, the blood glucose reduction might not be caused by sinensetin only because the water extract has an almost undetectable amount of sinensetin.<sup>14,24</sup>

Based on bioactivity-guided fractionation, the chloroform fraction of the plant exhibited a blood glucose-lowering effect in fasting, treated rats, when compared with controls, after glucose loading at 150 mg/kg.<sup>35</sup> Terpenoids and flavonoids, including sinensetin, were identified in the crude extract and the chloroform fraction. Hence, these compounds either acted separately or synergistically. Sinensetin and other compounds in the chloroform fraction were also found to be responsible for the antihyperglycemic effect.<sup>35</sup> Another team of researchers reported that the rosmarinic rich fraction of the ethanolic extract could achieve up to 100% inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase; this was about 5 times more active than the antidiabetic drug, acarbose.<sup>113</sup>

### Antioxidant Property of Plant Extract

Rosmarinic acid, which is an ester of caffeic acid with 3,4-dihydroxyphenylacetic acid (danshensu), is the characteristic plant constituent of *O. aristatus*.<sup>114</sup> Its content was found to be in the range of 5.1%-29.9% of the total dry leaf weight.<sup>15</sup> Rosmarinic acid is well known for its potential to improve insulin sensitivity, lower plasma lipid level,<sup>115</sup> and its antidiabetic effect.<sup>113,116</sup> The compound is also popular for its anti-inflammatory, antioxidative, antiviral, and antibacterial activities, which had been proven in many in vitro and in vivo studies.<sup>117</sup> In line with the review of Shahidi and Chandrasekara,<sup>118</sup> compounds with a hydroxycinnamyl group are potent antioxidants. Both caffeic acid and rosmarinic acid have a hydroxycinnamyl molecular structure with 2 and 4 hydroxyl groups, respectively. This explains the higher antioxidant activity of rosmarinic acid than caffeic acid.<sup>119</sup>

The pharmacological properties of the compounds are important to understand the wound-healing mechanism. Detailed studies demonstrated the inhibitory effects of rosmarinic acid on 5-lipoxygenase and 12-lipoxygenase and gene expression of cyclooxygenase-2 (COX-2).<sup>120-122</sup> The IC<sub>50</sub> value of the rosmarinic acid-rich extract on 5-lipoxygenase was 0.69  $\mu$ g/mL and 3.25  $\mu$ g/mL for p38 $\alpha$ ; the compound had only moderate inhibitory effects on TNF $\alpha$  release.<sup>117</sup> Moreover,

rosmarinic acid (5-20 mmol/L) showed an anti-inflammatory effect by reducing 12-O-tetradecanoylphorbol-13-acetate-induced COX-2 promoter activity and protein levels.<sup>120</sup>

*Orthosiphon stamineus* has also been proven to have excellent antioxidant properties. The methanol extract of the herb showed a variation in total phenolics ranging from 6.7 to 10.1 mg caffeic acid/g dry weight, in line with the antioxidant activities, which ranged from 55.5% to 84.2%. The antioxidative potency of the methanol extract was comparable to that of quercetin and the synthetic antioxidant, butylated hydroxyanisole (BHA).<sup>68</sup> Research conducted by Akowuah et al.<sup>32</sup> also found that different solvent systems with varied polarities resulted in different radical scavenging activities based on the in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The acetone extract showed the highest activity. The free radical-scavenging activities of the extracts were also comparable to those of quercetin and BHA.

The antioxidative potency of the methanol/water extract of *O. stamineus* has been demonstrated in terms of DPPH radical scavenging, Fe<sup>3+</sup> induced lipid peroxidation inhibition, and Trolox equivalent antioxidant capacity in in vitro models.<sup>123</sup> An aqueous extract of *O. stamineus* exhibited significant free radical scavenging activity with an IC<sub>50</sub> of 9.6  $\mu$ g/mL, whereas the IC<sub>50</sub> of a 50% ethanol extract was 21.4  $\mu$ g/mL. These results showed that *O. stamineus* possessed high antioxidant activity and could be considered as an immunomodulatory agent.<sup>30</sup>

Ultrasound-assisted extraction was used to extract antioxidant compounds from *O. stamineus*. The antioxidant activities of the extract were evaluated using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging and DPPH radical scavenging assays. The results were found to be 1961.3 and 2,423.3  $\mu$ mol Trolox Equivalent Antioxidant Capacity (TEAC)/100 g dry weight (DW), respectively. Rosmarinic acid, kaempferol-rutinoside, and sinensetin were identified by high-performance liquid chromatography-mass spectrometry in the study.<sup>124</sup> This shows again that the high antioxidative capacity of the plant extract could be due to the presence of these phenolic compounds.

Polymethoxy and polyhydroxy flavonoids were found to possess remarkable health-promoting benefits.<sup>125-127</sup> It is believed that most of the bioactivities are attributed to the chemical structure of the compounds, especially the polyhydroxyl groups as radical scavengers.<sup>128</sup> Polymethoxylated flavonoids are also readily absorbed in the intestine and have been shown to have a wide tissue distribution and metabolic stability.<sup>129</sup>

Polyhydroxy flavonoids were found to have better antioxidative activity than their monohydroxy derivatives. This is because polyhydroxy substituted flavonoids can easily donate electrons to scavenge free radicals and have remarkable inhibitory actions on lipid peroxidation and significantly to be more potent antioxidants, being about 2.5-4 times higher than the reference compound Trolox.<sup>127</sup>

The polymethoxylated and/or polyhydroxylated flavonoids in *O. aristatus* are sinensetin, eupatorin, and 3'-hydroxy-5,6,7,4'-tetra-methoxyflavone (TMF). These compounds were found to be the dominant compounds in the chloroform fractions.<sup>69</sup>

and were chosen as markers to standardize various leaf extracts.<sup>16,24,68,103,130</sup> Eupatorin and sinensetin were found to have in vivo anti-inflammatory properties. The expression of inflammatory genes such as inducible nitric oxide synthase (iNOS), COX-2, and TNF- $\alpha$ , as well as the production of inflammatory mediators like nitric oxide (NO) and prostaglandin E2 (PGE2), were suppressed in vitro, probably by inhibiting the enzyme activity and the activity of a transcription factor STAT1 $\alpha$ .<sup>131</sup>

Moreover, an ethanolic extract of *O. stamineus* containing 1.02% TMF, 3.76% sinensetin, and 3.03% eupatorin possessed an inhibitory activity toward  $\alpha$ -glucosidase.<sup>132</sup> In addition, the 50% ethanolic extract of *O. stamineus*, and isolated sinensetin, were shown to have equal preference for both  $\alpha$ -glucosidase and  $\alpha$ -amylase. The 50% inhibitory activities were 4.63 and 0.66 mg/mL, respectively for  $\alpha$ -glucosidase, and 36.7 mg/mL and 1.13 mg/mL, respectively for  $\alpha$ -amylase.<sup>66</sup> Therefore, the presence of polymethoxylated and/or polyhydroxylated flavonoids is very important for the potential of the plant extract as a DFU remedy.

#### *Anti-inflammatory Property of Plant Extract*

Previous in vitro and in vivo studies revealed that *O. aristatus* possesses remarkable anti-inflammatory activity.<sup>131</sup> Its ethanolic extract inhibited lipopolysaccharide (LPS)-stimulated NO, PGE2, and intracellular ROS production in RAW 264.7 cells. Moreover, the plant extract was able to inhibit protein and messenger ribonucleic acid expression of iNOS and COX-2 in LPS-stimulated RAW 264.7 cells. Ursolic acid, detected in the extract by high-performance liquid chromatography, was shown to suppress LPS-induced NO and PGE2 production by inhibiting ROS generation, along with reducing the expression of iNOS and COX-2 in RAW 264.7 cells. Therefore, the ethanol extract of *O. aristatus* is likely to have promising effects on the metabolic pathway of inflammatory-mediated diseases.<sup>133</sup>

The NO inhibitory activity was probably due to the presence of 47 diterpenes isolated from *O. stamineus*.<sup>134</sup> All the diterpenes displayed significant concentration-dependent inhibition of NO production in macrophage-like J774.1 cells. The activities of the compounds varied depending upon the chemical structure. Although NO is an important signaling molecule, its excessive production triggers tissue damage and the release of proinflammatory cytokines such as tumor necrosis factor, interferon, and interleukin-1.<sup>71</sup>

The findings reported by Yam et al.<sup>70</sup> justified the traditional use of *O. aristatus* in treating pain and inflammation. The 50% methanol extract of *O. stamineus* was found to possess anti-inflammatory and analgesic activities. Oral administration at doses of 500 and 1000 mg/kg significantly reduced hind paw edema in rats at 3 and 5 hours after carrageenan administration and produced significant analgesic activity ( $P < 0.05$ ) in both the acetic acid-induced writhing test and the formalin-induced licking test.

#### **No Adverse Effects of Plant Extract**

Till now, no sign of toxicity has been reported in the literature for *O. aristatus* extract. Previously, 4 test groups of female Sprague-Dawley rats were treated up to 14 days with a methanolic extract of the plant at concentrations from 0.5 to 5 g/kg body weight.<sup>135</sup> No lethality nor adverse toxic signs were noticed during the experimental period. Abdullah et al.<sup>136</sup> also reported similar results, with no death record. The animals that were administered with 5000 mg/kg body weight of the plant extract did not show signs of toxicity during the experimental period. The median lethal dose (LD<sub>50</sub>) was estimated to be more than 5000 mg/kg body weight, with no relative change in the general behavior, body weight, food and water intake, relative organ weight per 100 g body weight, and hematological and clinical biochemistry analyses.<sup>136</sup> The methanolic extract of *O. aristatus* was also reported to produce no sign of toxicity.<sup>36</sup> With oral administration at doses of 1250, 2500, and 5000 mg/kg/day for 28 days, no abnormality of internal organs was observed between the treatment and control groups. The oral lethal dose was more than 5000 mg/kg and the no-observed-adverse-effect level (NOAEL) of the methanol extract for both male and female rats was considered to be 5000 mg/kg/day.<sup>36,45</sup> The standardized aqueous extract with a concentration up to 2000 mg/kg/day did not alter pregnancy body weight gain, food and water consumption, and any other sign of maternal toxicity.<sup>137</sup> Therefore, *O. aristatus* extract was considered to have a NOAEL of up to 5000 mg/kg/day.

#### **Conclusion**

DFUs continue to be a significant burden for people with diabetes, caregivers, and the health care system. Traditional Chinese medicines have been used for diabetes treatment and antidiabetic foot ulcer with proven clinical efficacy. The effectiveness was associated with the normalization of glycemic control in diabetes. Thus, proper management of blood glucose levels is the key factor to heal DFUs. Many phytochemicals in *O. aristatus* have been postulated to promote DFU healing, mainly due to their capability of lowering blood glucose. The presence of compounds such as phenolic acids, flavonoids, and triterpenes are postulated to be involved in the 4 phases of wound healing, due to their remarkable antioxidant, anti-inflammatory, and antihyperglycemic properties. No adverse toxic effect of the plant extract was observed and the LD<sub>50</sub> was estimated to be more than 5000 mg/kg body weight. Therefore, *O. aristatus* extract could be a potential DFU phytomedicine for further pre-clinical and clinical studies.

#### **Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

#### **Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors thank the Ministry of Higher Education, Malaysia,



and Universiti Teknologi Malaysia for giving the research grant of HICoE-4J263 and TDR-07G21.

## ORCID ID

Lee Suan Chua  <https://orcid.org/0000-0003-2493-9963>

## References

- Cragg GM, Newman DJ. Drugs from nature: past achievements, future prospects. In: *Advances in Phytomedicine*. 1. Elsevier; 2002:23-37.
- Heinrich M, Barnes J, Gibbons S, Williamson EM. *Fundamentals of Pharmacognosy and Phytotherapy E-Book*. Elsevier Health Sciences; 2012.
- Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect*. 2001;109(1):69-75. doi:10.1289/ehp.01109s169
- Getahun T, Sharma V, Gupta N. The genus *Laggera* (Asteraceae)–ethnobotanical and ethnopharmacological information, chemical composition as well as biological activities of its essential oils and extracts: A review. *Chem Biodivers*. 2019;16(8):e1900131. doi:10.1002/cbdv.201900131
- Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. *Evidence-Based Complement Altern Med*. 2019;2019(2):1-30. doi:10.1155/2019/2684108
- Pan Y, Abd-Rashid BA, Ismail Z, et al. In vitro modulatory effects of *Andrographis paniculata*, *Centella asiatica* and *Orthosiphon stamineus* on cytochrome P450 2C19 (CYP2C19). *J Ethnopharmacol*. 2011;133(2):881-887. doi:10.1016/j.jep.2010.11.026
- Tezuka Y, Stampoulis P, Banskota AH, et al. Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*. *Chem Pharm Bull*. 2000;48(11):1711-1719. doi:10.1248/cpb.48.1711
- Tran K. *Medicinal Plants in Vietnam*. WHO Regional Office for the Western Pacific Manila, and Institute of Materia Medica Hanoi, Science and Technology Publishing House; 1970.
- Awale S, Tezuka Y, Kobayashi M, Ueda JY, Kadota S. Neoorthosiphonone A; a nitric oxide (NO) inhibitory diterpene with new carbon skeleton from *Orthosiphon stamineus*. *Tetrahedron Lett*. 2004;45(7):1359-1362. doi:10.1016/j.tetlet.2003.12.054
- Nguyen MTT, Awale S, Tezuka Y, Chien-Hsiung C, Kadota S. Staminane- and isopimarane-type diterpenes from *Orthosiphon stamineus* of Taiwan and their nitric oxide inhibitory activity. *J Nat Prod*. 2004;67(4):654-658. doi:10.1021/np030471+
- Arafat OM, Tham SY, Sadikun A, Zhari I, Haughton PJ, Asmawi MZ. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats. *J Ethnopharmacol*. 2008;118(3):354-360. doi:10.1016/j.jep.2008.04.015
- Basheer M, Majid A. Medicinal potentials of Benth. *Webmed Cent*. 2010;1(12):1-7.
- Ashraf K, Sultan S, Adam A. *Orthosiphon stamineus* Benth is an outstanding food medicine: review of phytochemical and pharmacological activities. *J Pharm Bioallied Sci*. 2017;10(3):1-5.
- Pang SF, Lau MZ, Yusoff MM, Gimbin J. Microwave-irradiation induced fast simultaneous extraction of methoxylated and hydroxylated phenolic compounds from *Orthosiphon stamineus* leaves. *Mater Sci Forum*. 2017;890:155-158. doi:10.4028/www.scientific.net/MSF.890.155
- Han CJ. Drug metabolism and toxicity studies of *Orthosiphon stamineus* Benth (Misai Kucing) in rats. 2006.
- Hamil MS, Ismail Z, Abdul Majid AMS, Saidan N, Aisha AA. A novel reverse phase high-performance liquid chromatography method for standardization of *Orthosiphon stamineus* leaf extracts. *Pharmacognosy Res*. 2014;7(1):23.
- Nuengchamnon N, Krittasilp K, Ingkaninan K. Characterisation of phenolic antioxidants in aqueous extract of *Orthosiphon grandiflorus* tea by LC-ESI-MS/MS coupled to DPPH assay. *Food Chem*. 2011;127(3):1287-1293. doi:10.1016/j.foodchem.2011.01.085
- Siddiqui MJA, Ismail Z. Simultaneous analysis of bioactive markers from *Orthosiphon stamineus* Benth leaves extracts by reverse phase high performance liquid chromatography. *Trop J Pharm Res*. 2011;10(1):97-103. doi:10.4314/tjpr.v10i1.66548
- Wray V, Sumaryono W, Hartmann T, Witte L, Proksch P. Qualitative and quantitative analysis of the phenolic constituents from *Orthosiphon aristatus*. *Planta Med*. 2007;73(02):176-180.
- Hossain MA, Ismail Z. Isolation and characterization of triterpenes from the leaves of *Orthosiphon stamineus*. *Arab J Chem*. 2013;6(3):295-298. doi:10.1016/j.arabj.2010.10.009
- Malterud KF, Hanche-Olsen IM, Smith-Kielland I. Flavonoids from *Orthosiphon spicatus*. *Planta Med*. 1989;55(6):569-570. doi:10.1055/s-2006-962099
- Pietta PG, Mauri PL, Gardana C, Bruno A. High-performance liquid chromatography with diode-array ultraviolet detection of methoxylated flavones in *Orthosiphon* leaves. *J Chromatogr A*. 1991;547(C):439-442. doi:10.1016/S0021-9673(01)88668-4
- Awale S, Tezuka Y, Banskota AH, Kouda K, Tun KM, Kadota S. Four highly oxygenated isopimarane-type diterpenes of *Orthosiphon stamineus*. *Planta Med*. 2002;68(3):286-288. doi:10.1055/s-2002-23137
- Gimbin J, Pang SF, Yusoff MM. *Orthosiphon stamineus* (Java tea). Elsevier Inc; 2018.
- Awale S, Tezuka Y, Banskota AH, Kouda K, Tun KM, Kadota S. Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. *J Nat Prod*. 2001;64(5):592-596. doi:10.1021/np000607t
- Akowuah GA, Zhari I. Effect of extraction temperature on stability of major polyphenols and antioxidant activity of *Orthosiphon stamineus* leaf. *J Herbs Spices Med Plants*. 2010;16(3-4):160-166. doi:10.1080/10496475.2010.509652
- Pang SF, Yusoff MM, Gimbin J. Assessment of phenolic compounds stability and retention during spray drying of *Orthosiphon stamineus* extracts. *Food Hydrocoll*. 2014;37:159-165. doi:10.1016/j.foodhyd.2013.10.022
- Hossain MA, Ismail Z, Rahman A, Kang SC. Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. *Ind Crops Prod*. 2008;27(3):328-334. doi:10.1016/j.indcrop.2007.11.008
- Ho CH, Noryati I, Sulaiman SF, Rosma A. In vitro antibacterial and antioxidant activities of *Orthosiphon stamineus* Benth. extracts against food-borne bacteria. *Food Chem*. 2010;122(4):1168-1172.

30. Alshawsh MA, Abdulla MA, Ismail S, et al. Free radical scavenging, antimicrobial and immunomodulatory activities of *Orthosiphon stamineus*. *Molecules*. 2012;17(5):5385-5395. doi:10.3390/molecules17055385
31. Reena L, Devi MD, Singh SR. Anti-bacterial efficacy of elite medicinal plants on urolithiasis inducing flora. *J Food, Agric Environ*. 2009;7(2):40-45.
32. Akowuah GA, Ismail Z, Norhayati I, Sadikun A. The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chem*. 2005;93(2):311-317. doi:10.1016/j.foodchem.2004.09.028
33. Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite profiling and wound-healing activity of *Boerhavia diffusa* leaf extracts using in vitro and in vivo models. *J Tradit Complement Med*. 2020;10(1):52-59. doi:10.1016/j.jtcme.2019.02.002
34. Sekar M, Abdullah MZ, Nor Azlan AYH, Nasir SN, Zakaria Z, Abdullah MS. Ten commonly available medicinal plants in Malaysia used for the treatment of diabetes - A review. *Asian J Pharm Clin Res*. 2014;7(1):1-5.
35. Mohamed EAH, Mohamed AJ, Asmawi MZ, Sadikun A, Ebrika OS, Yam MF. Antihyperglycemic effect of *Orthosiphon stamineus* Benth leaves extract and its bioassay-guided fractions. *Molecules*. 2011;16(5):3787-3801. doi:10.3390/molecules16053787
36. Yam MF, Lim CP, Fung Ang L, et al. Antioxidant and toxicity studies of 50% methanolic extract of *Orthosiphon stamineus* Benth. *Biomed Res Int*. 2013;2013:1-10. doi:10.1155/2013/351602
37. Lokman EF, Saparuddin F, Muhammad H, Omar MH, Zulkapli A. *Orthosiphon stamineus* as a potential antidiabetic drug in maternal hyperglycemia in streptozotocin-induced diabetic rats. *Integr Med Res*. 2019;8(3):173-179. doi:10.1016/j.imr.2019.05.006
38. Mohamed EAH, Siddiqui MJA, Ang LF, et al. Potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. *BMC Complement Altern Med*. 2012;12(1):176. doi:10.1186/1472-6882-12-176
39. Ching SM, Zakaria ZA, Paimin F, Jalalian M. Complementary alternative medicine use among patients with type 2 diabetes mellitus in the primary care setting: a cross-sectional study in Malaysia. *BMC Complement Altern Med*. 2013;13(1):1-7. doi:10.1186/1472-6882-13-148
40. Dreifke MB, Jayasuriya AA, Jayasuriya AC. Current wound healing procedures and potential care. *Mater Sci Eng C*. 2015;48:651-662. doi:10.1016/j.msec.2014.12.068
41. Fletcher J, Wounds S. *Lower Extremity Wounds: A Problem-Based Learning Approach*. 23. Oxford University Press; 2008:139-160.
42. Wang J, Windbergs M. Functional electrospun fibers for the treatment of human skin wounds. *Eur J Pharm Biopharm*. 2017;119:283-299. doi:10.1016/j.ejpb.2017.07.001
43. Korting HC, Schöllmann C, White RJ. Management of minor acute cutaneous wounds: importance of wound healing in a moist environment. *J Eur Acad Dermatology Venereol*. 2011;25(2):130-137. doi:10.1111/j.1468-3083.2010.03775.x
44. Alam G, Singh MP, Singh A. Wound healing potential of some medicinal plants. *Int J Pharm Sci Rev Res*. 2011;9:136-145.
45. Karri V, Kuppasamy G, Talluri SV, et al. Curcumin loaded chitosan nanoparticles impregnated into collagen-alginate scaffolds for diabetic wound healing. *Int J Biol Macromol*. 2016;93(Pt B):1519-1529. doi:10.1016/j.ijbiomac.2016.05.038
46. Tam JCW, Lau KM, Liu CL, et al. The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action. *J Ethnopharmacol*. 2011;134(3):831-838. doi:10.1016/j.jep.2011.01.032
47. Singh S, Young A, McNaught CE. The physiology of wound healing. *Surg (United Kingdom)*. 2017;35(9):473-477. doi:10.1016/j.mpsur.2017.06.004
48. Martin P. Wound healing - Aiming for perfect skin regeneration. *Science (80-)*. 1997;276(5309):75-81. doi:10.1126/science.276.5309.75
49. Shukla R, Kashaw SK, Jain AP, Lodhi S. Fabrication of apigenin loaded gellan gum-chitosan hydrogels (GGCH-HGs) for effective diabetic wound healing. *Int J Biol Macromol*. 2016;91:1110-1119. doi:10.1016/j.ijbiomac.2016.06.075
50. Vig K, Chaudhari A, Tripathi S, et al. Advances in skin regeneration using tissue engineering. *Int J Mol Sci*. 2017;18(4):789. doi:10.3390/ijms18040789
51. Geethalakshmi R, Sakravarthi C, Kritika T, Arul Kirubakaran M, Sarada DVL. Evaluation of antioxidant and wound healing potentials of *Sphaeranthus amaranthoides* Burm. *Biomed Res Int*. 2013;2013(4):1-7. doi:10.1155/2013/607109
52. Moura LIF, Dias AMA, Carvalho E, De Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment - a review. *Acta Biomater*. 2013;9(7):7093-7114. doi:10.1016/j.actbio.2013.03.033
53. Ekmektzoglou KA, Zografos GC. A concomitant review of the effects of diabetes mellitus and hypothyroidism in wound healing. *World J Gastroenterol WJG*. 2006;12(17):2721-2729. doi:10.3748/wjg.v12.i17.2721
54. Fernando ME, Seneviratne RM, Cunningham M, et al. Intensive versus conventional glycaemic control for treating diabetic foot ulcers. *Cochrane Database Syst Rev*. 2013;2013(10).
55. Schaper NC. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab Res Rev*. 2004;20(S1):90-S95. doi:10.1002/dmrr.464
56. Rafehi H, El-Osta A, Karagiannis TC. Genetic and epigenetic events in diabetic wound healing. *Int Wound J*. 2011;8(1):12-21. doi:10.1111/j.1742-481X.2010.00745.x
57. Narayanan SK. A multifactorial approach to targeting signalling pathways in diabetic foot ulcers. 2019.
58. Chin Y-F, Huang T-T, Hsu BR-S, Weng L-C, Wang C-C. Factors associated with foot ulcer self-management behaviours among hospitalised patients with diabetes. *J Clin Nurs*. 2019;28(11-12):2253-2264. doi:10.1111/jocn.14822
59. Morey M, O'Gaora P, Pandit A, Hélarly C. Hyperglycemia acts in synergy with hypoxia to maintain the pro-inflammatory

- phenotype of macrophages. *PLoS One*. 2019;14(8):e0220577. doi:10.1371/journal.pone.0220577
60. Luong KVQ, Nguyen LTH. The impact of thiamine treatment in the diabetes mellitus. *J Clin Med Res*. 2012;4(3):153-160. doi:10.4021/jocmr890w
61. Panda S, Gupta SK, Singh SK. The etiopathogenesis of the diabetic foot: an unrelenting epidemic. *Int J Low Extrem Wounds*. 2010;9(3):127-131. doi:10.1177/1534734610380029
62. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005;54(6):1615-1625. doi:10.2337/diabetes.54.6.1615
63. Giacco F, Brownlee M. Mechanisms of hyperglycemic damage in diabetes. In: *Atlas of Diabetes*. Springer; 2012:217-231.
64. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010;107(9):1058-1070. doi:10.1161/CIRCRESAHA.110.223545
65. D'Souza DR, Salib MM, Bennett J, et al. Hyperglycemia regulates RUNX2 activation and cellular wound healing through the aldose reductase polyol pathway. *J Biol Chem*. 2009;284(27):17947-17955. doi:10.1074/jbc.M109.002378
66. Mohamed EAH, Siddiqui MJA, Ang LF, et al. Potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. *BMC Complement Altern Med*. 2012;12(1) doi:10.1186/1472-6882-12-176
67. Ahmad FB, Holdsworth DK, Sekar M, et al. Ten commonly available medicinal plants in Malaysia used for the treatment of diabetes - A review. *Asian J Pharm Clin Res*. 2014;7(5):378-383.
68. Akowuah GA, Zhari I, Norhayati I, Sadikun A, Khamsah SM. Sinensetin, eupatorin, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. *Food Chem*. 2004;87(4):559-566. doi:10.1016/j.foodchem.2004.01.008
69. Yam MF, Lim V, Salman IM, et al. HPLC and anti-inflammatory studies of the flavonoid rich chloroform extract fraction of *Orthosiphon stamineus* leaves. *Molecules*. 2010;15(6):4452-4466. doi:10.3390/molecules15064452
70. Yam MF, Asmawi MZ, Basir R. An investigation of the anti-inflammatory and analgesic effects of *Orthosiphon stamineus* leaf extract. *J Med Food*. 2008;11(2):362-368. doi:10.1089/jmf.2006.065
71. Kuo PC, Schroeder RA. The emerging multifaceted roles of nitric oxide. *Ann Surg*. 1995;221(3):220-235. doi:10.1097/0000658-199503000-00003
72. Idris I, Game F, Jeffcoate W. Does close glycaemic control promote healing in diabetic foot ulcers? Report of a feasibility study. *Diabet Med*. 2005;22(8):1060-1063. doi:10.1111/j.1464-5491.2005.01606.x
73. Estelle E, Mathioudakis N. Update on management of diabetic foot ulcers. *Physiol Behav*. 2017;176(3):139-148.
74. Christman AL, Selvin E, Margolis DJ, Lazarus GS, Garza LA. Hemoglobin A1c predicts healing rate in diabetic wounds. *J Invest Dermatol*. 2011;131(10):2121-2127. doi:10.1038/jid.2011.176
75. Markuson M, Hanson D, Anderson J, et al. The relationship between hemoglobin A1c values and healing time for lower extremity ulcers in individuals with diabetes. *Adv Skin Wound Care*. 2009;22(8):365-372. doi:10.1097/01.ASW.0000358639.45784.cd
76. Fernando ME, Seneviratne RM, Tan YM, et al. Intensive versus conventional glycaemic control for treating diabetic foot ulcers. *Cochrane Database Syst Rev*. 2016;1:CD010764. doi:10.1002/14651858.CD010764.pub2
77. Hemmingsen B, Lund SS, Glud C, et al. Targeting intensive glycaemic control versus targeting conventional glycaemic control for type 2 diabetes mellitus. *Cochrane Database Syst Rev*. 2013;11:1-173. doi:10.1002/14651858.CD008143.pub3
78. Matilla TK, de Boer A. Influence of intensive versus conventional glucose control on microvascular and macrovascular complications in type 1 and 2 diabetes mellitus. *Drugs*. 2010;70(17):2229-2245. doi:10.2165/11585220-000000000-00000
79. Loots MAM, Lamme EN, Mekkes JR, Bos JD, Middelkoop E. Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. *Arch Dermatol Res*. 1999;291(2-3):93-99. doi:10.1007/s004030050389
80. Spravchikov N, Sizyakov G, Gartsbein M, Accili D, Tennenbaum T, Wertheimer E. Glucose effects on skin keratinocytes: implications for diabetes skin complications. *Diabetes*. 2001;50(7):1627-1635. doi:10.2337/diabetes.50.7.1627
81. Pierre EJ, Perez-Polo JR, Mitchell AT, Matin S, Foyt HL, Herndon DN. Insulin-like growth factor-I liposomal gene transfer and systemic growth hormone stimulate wound healing. *J Burn Care Rehabil*. 1997;18(4):287-291. doi:10.1097/00004630-199707000-00002
82. Weringer EJ, Kelso JM, Tamai IY, Arquilla ER. The effect of antisera to insulin, 2-deoxyglucose-induced hyperglycemia, and starvation on wound healing in normal mice. *Diabetes*. 1981;30(5):407-410. doi:10.2337/diab.30.5.407
83. Soleimani Z, Hashemdokht F, Bahmani F, Taghizadeh M, Memarzadeh MR, Asemi Z. Clinical and metabolic response to flaxseed oil omega-3 fatty acids supplementation in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. *J Diabetes Complications*. 2017;31(9):1394-1400. doi:10.1016/j.jdiacomp.2017.06.010
84. Ko CH, Yi S, Ozaki R, et al. Healing effect of a two-herb recipe (NF3) on foot ulcers in Chinese patients with diabetes: a randomized double-blind placebo-controlled study. *J Diabetes*. 2014;6(4):323-334. doi:10.1111/1753-0407.12117
85. Pharmacopoeia N. *Pharmacopoeia of the People's Republic of China*. China People's Med Publ House; 2005.
86. Lau CH, Chan CM, Chan YW, et al. In vitro antidiabetic activities of five medicinal herbs used in Chinese medicinal formulae. *Phytother Res*. 2008;22(10):1384-1388. doi:10.1002/ptr.2513
87. Wong MW, Leung PC, Wong WC. Limb salvage in extensive diabetic foot ulceration-a preliminary clinical study using simple debridement and herbal drinks. *Hong Kong Med J*. 2001;7(4):403-407.

88. Amzad Hossain M, Ismail Z. Quantification and enrichment of sinensetin in the leaves of *Orthosiphon stamineus*. *Arab J Chem*. 2016;9:S1338-S1341. doi:10.1016/j.arabjc.2012.02.016
89. Tsuchiya H, Sato M, Miyazaki T, et al. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J Ethnopharmacol*. 1996;50(1):27-34. doi:10.1016/0378-8741(96)85514-0
90. Villegas LF, Fernández ID, Maldonado H, et al. Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. *J Ethnopharmacol*. 1997;55(3):193-200. doi:10.1016/S0378-8741(96)01500-0
91. Guimarães AC, Meireles LM, Lemos MF, et al. Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*. 2019;24(13):1-12. doi:10.3390/molecules24132471
92. Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK. Antiulcer and in vitro antioxidant activities of *Jasminum grandiflorum* L. *J Ethnopharmacol*. 2007;110(3):464-470. doi:10.1016/j.jep.2006.10.017
93. Rodríguez JA, Astudillo L, Schmeda-Hirschmann G. Oleanolic acid promotes healing of acetic acid-induced chronic gastric lesions in rats. *Pharmacol Res*. 2003;48(3):291-294. doi:10.1016/S1043-6618(03)00155-5
94. Mukherjee H, Ojha D, Bharitkar YP, et al. Evaluation of the wound healing activity of *Shorea robusta*, an Indian ethnomedicine, and its isolated constituent(s) in topical formulation. *J Ethnopharmacol*. 2013;149(1):335-343. doi:10.1016/j.jep.2013.06.045
95. Byun-McKay A, Godard K-A, Toudefallah M, et al. Wound-induced terpene synthase gene expression in Sitka spruce that exhibit resistance or susceptibility to attack by the white pine weevil. *Plant Physiol*. 2006;140(3):1009-1021. doi:10.1104/pp.105.071803
96. Song HS, Park TW, Sohn UD, et al. The effect of caffeic acid on wound healing in skin-incised mice. *Korean J Physiol Pharmacol*. 2008;12(6):343-347. doi:10.4196/kjpp.2008.12.6.343
97. Agra LC, Ferro JNS, Barbosa FT, Barreto E. Triterpenes with healing activity: a systematic review. *J Dermatolog Treat*. 2015;26(5):465-470. doi:10.3109/09546634.2015.1021663
98. Abdelwahab SI, Mohan S, Mohamed Elhassan M, et al. Antiapoptotic and antioxidant properties of *Orthosiphon stamineus* Benth (Cat's whiskers): intervention in the Bcl-2-mediated apoptotic pathway. *Evid Based Complement Altern Med*. 2011;2011(13):156765. doi:10.1155/2011/156765
99. Chan KWK, Ho WS. Anti-oxidative and hepatoprotective effects of lithospermic acid against carbon tetrachloride-induced liver oxidative damage in vitro and in vivo. *Oncol Rep*. 2015;34(2):673-680. doi:10.3892/or.2015.4068
100. Xiao H, Xie G, Wang J, et al. Chicoric acid prevents obesity by attenuating hepatic steatosis, inflammation and oxidative stress in high-fat diet-fed mice. *Food Res Int*. 2013;54(1):345-353. doi:10.1016/j.foodres.2013.07.033
101. Chen YS, Lee SM, Lin YJ, Chiang SH, Lin CC. Effects of danshensu and salvianolic acid B from *Salvia miltiorrhiza* Bunge (Lamiaceae) on cell proliferation and collagen and melanin production. *Molecules*. 2014;19(2):2029-2041. doi:10.3390/molecules19022029
102. Indariani S, Hanny Wijaya C, Rahminiwati M, Wien Winarno M. Antihyperglycemic activity of functional drinks based on java tea (*Orthosiphon aristatus*) in streptozotocin induced diabetic mice. *Int Food Res J*. 2014;21(1):349-355.
103. Yam MF, Mohamed EAH, Ang LF, et al. A Simple isocratic HPLC method for the simultaneous determination of sinensetin, eupatorin, and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone in *Orthosiphon stamineus* extracts. *JAMS J Acupunct Meridian Stud*. 2012;5(4):176-182. doi:10.1016/j.jams.2012.05.005
104. Stampoulis P, Tezuka Y, Banskota AH, Saiki I, Kadola S, Staminol A, a novel diterpene from *Orthosiphon stamineus*. *Tetrahedron Lett*. 1999;40(22):4239-4242. doi:10.1016/S0040-4039(99)00685-1
105. Awale S, Tezuka Y, Banskota AH, Adnyana IK, Kadota S. Nitric oxide inhibitory isopimarane-type diterpenes from *Orthosiphon stamineus* of Indonesia. *J Nat Prod*. 2003;66(2):255-258. doi:10.1021/np020455x
106. Ohashi K, Bohgaki T, Matsubara T, Shibuya H. Indonesian medicinal plants. XXIII. Chemical structures of two new migrated pimarane-type diterpenes, neoorthosiphols A and B, and suppressive effects on rat thoracic aorta of chemical constituents isolated from the leaves of *Orthosiphon aristatus* (Lamiaceae). *Chem Pharm Bull*. 2000;48(3):433-435. doi:10.1248/cpb.48.433
107. Shibuya M, Hoshino M, Katsube Y, Hayashi H, Kushiro T, Ebizuka Y. Identification of  $\beta$ -amyrin and sophoradiol 24-hydroxylase by expressed sequence tag mining and functional expression assay. *FEBS J*. 2006;273(5):948-959. doi:10.1111/j.1742-4658.2006.05120.x
108. Guerin JC, Reveillere HP, Ducrey P, Toupet L. *Orthosiphon stamineus* as a potent source of methylripariochromene A. *J Nat Prod*. 1989;52(1):171-173. doi:10.1021/np50061a023
109. Mariam A, Asmawi MZ, Sadikun A. Hypoglycaemic activity of the aqueous extract of *Orthosiphon stamineus*. *Fitoterapia*. 1996;67(5):465-468.
110. Sriplang K, Adisakwattana S, Rungsipipat A, Yibchok-anun S. Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 2007;109(3):510-514. doi:10.1016/j.jep.2006.08.027
111. Adnyana K, Setiawan F, Insanu M. From ethnopharmacology to clinical study of *Orthosiphon stamineus* Benth. *Int J Pharm Pharm Sci*. 2013;5(3):66-73.
112. Singh MK, Gidwani B, Gupta A, Dhongade H, Kashyap PP, Tripathi DK. A review of the medicinal plants of genus *Orthosiphon* (Lamiaceae). *Int J. Biol Chem*. 2015;9(6):318-331. doi:10.3923/ijbc.2015.318.331
113. Ngo YL, Chua LS. Anti-diabetic activity of rosmarinic acid rich fractions from *Orthosiphon stamineus*. *Curr Enzym Inhib*. 2018;14(2):97-103.
114. Trute A, Nahrstedt A. Separation of rosmarinic acid enantiomers by three different chromatographic methods (HPLC, CE, GC) and the determination of rosmarinic acid in *Hedera helix* L. *Phytochem Anal*. 1996;7(4):204-208.

- doi:10.1002/(SICI)1099-1565(199607)7:4<204::AID-PCA301>3.0.CO;2-4
115. Karthik D, Viswanathan P, Anuradha CV. Administration of rosmarinic acid reduces cardiopathology and blood pressure through inhibition of p22phox NADPH oxidase in fructose-fed hypertensive rats. *J Cardiovasc Pharmacol.* 2011;58(5):514-521. doi:10.1097/FJC.0b013e31822c265d
  116. Berhow MA, Affum AO, Gyan BA. Rosmarinic acid content in antidiabetic aqueous extract of *Ocimum canum* Sims grown in Ghana. *J Med Food.* 2012;15(7):611-620. doi:10.1089/jmf.2011.0278
  117. Geller F, Schmidt C, Götttert M, et al. Identification of rosmarinic acid as the major active constituent in *Cordia americana*. *J Ethnopharmacol.* 2010;128(3):561-566. doi:10.1016/j.jep.2010.01.062
  118. Shahidi F, Chandrasekara A. Hydroxycinnamates and their in vitro and in vivo antioxidant activities. *Phytochem Rev.* 2010;9(1):147-170. doi:10.1007/s11101-009-9142-8
  119. Chen JH, Ho C-T. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J Agric Food Chem.* 1997;45(7):2374-2378. doi:10.1021/jf970055t
  120. Scheckel KA, Degner SC, Romagnolo DF. Rosmarinic acid antagonizes activator protein-1-dependent activation of cyclooxygenase-2 expression in human cancer and nonmalignant cell lines. *J Nutr.* 2008;138(11):2098-2105. doi:10.3945/jn.108.090431
  121. Psotová J, Kolár M, Soušek J, Svagera Z, Vičar J, Ulrichová J. Biological activities of *Prunella vulgaris* extract. *Phyther Res An Int J Devoted to Pharmacol Toxicol Eval Nat Prod Deriv.* 2003;17(9):1082-1087. doi:10.1002/ptr.1324
  122. Yamamoto H, Sakakibara J, Nagatsu A, Sekiya K. Inhibitors of arachidonate lipoxygenase from defatted perilla seed. *J Agric Food Chem.* 1998;46(3):862-865. doi:10.1021/jf970520m
  123. Yam MF, Basir R, Asmawi MZ, Ismail Z. Antioxidant and hepatoprotective effects of *Orthosiphon stamineus* Benth. standardized extract. *Am J Chin Med.* 2007;35(1):115-126. doi:10.1142/S0192415X07004679
  124. Ho SK, Tan CP, Thoo YY, Abas F, Ho CW. Ultrasound-assisted extraction of antioxidants in Misai Kucing (*Orthosiphon stamineus*). *Molecules.* 2014;19(8):12640-12659. doi:10.3390/molecules190812640
  125. Li X-L, Zhang Y-H, Wang C-F, Wang Q-A. Synthesis and antiproliferative activities of aminoalkylated polymethoxyflavonoid derivatives. *Nat Prod Res.* 2019;33(6):827-834. doi:10.1080/14786419.2017.1413562
  126. Banerji A. Bioinspired syntheses of partially methylated flavonoids—untapped source of bioactivities. *J Explor Res Pharmacol.* 2017;1(2):16-20. doi:10.14218/JERP.2016.00027
  127. Pahari B, Chakraborty S, Chaudhuri S, Sengupta B, Sengupta PK. Binding and antioxidant properties of therapeutically important plant flavonoids in biomembranes: insights from spectroscopic and quantum chemical studies. *Chem Phys Lipids.* 2012;165(4):488-496. doi:10.1016/j.chemphyslip.2011.10.006
  128. Chen S, Li X, Liu X, et al. Investigation of chemical composition, antioxidant activity, and the effects of Alfalfa flavonoids on growth performance. *Oxid Med Cell Longev.* 2020;2020:1-11. doi:10.1155/2020/8569237
  129. Ruiu S, Anzani N, Orrü A, et al. Methoxyflavones from *Stachys glutinosa* with binding affinity to opioid receptors: in silico, in vitro, and in vivo studies. *J Nat Prod.* 2015;78(1):69-76. doi:10.1021/np500671v
  130. Hashim S, Beh H, Hamil M, Ismail Z, Majid A. High-performance thin-layer chromatography method development, validation, and simultaneous quantification of four compounds identified in standardized extracts of *Orthosiphon stamineus*. *Pharmacogn Res.* 2016;8(4):238-243. doi:10.4103/0974-8490.188872
  131. Laavola M, Nieminen R, Yam M, et al. Flavonoids eupatorin and sinensetin present in *Orthosiphon stamineus* leaves inhibit inflammatory gene expression and STAT1 activation. *Planta Med.* 2012;78(8):779-786. doi:10.1055/s-0031-1298458
  132. Mohamed EA, Ahmad M, Ang LF, Asmawi MZ, Yam MF. Evaluation of  $\alpha$ -glucosidase inhibitory effect of 50% ethanolic standardized extract of *Orthosiphon stamineus* Benth in normal and streptozotocin-induced diabetic rats. *Evidence-based Complement Altern Med.* 2015;2015(3):1-6. doi:10.1155/2015/754931
  133. Hsu CL, Hong BOH, Yu Y-S, Yen G-C. Antioxidant and anti-inflammatory effects of *Orthosiphon aristatus* and its bioactive compounds. *J Agric Food Chem.* 2010;58(4):2150-2156. doi:10.1021/jf903557c
  134. Awale S, Tezuka Y, Banskota AH, Kadota S. Inhibition of NO production by highly-oxygenated diterpenes of *Orthosiphon stamineus* and their structure-activity relationship. *Biol Pharm Bull.* 2003;26(4):468-473. doi:10.1248/bpb.26.468
  135. Chin JH, Abas HH, Sabariah I, Han CJ, Hussin AH, Ismail S. Toxicity study of *Orthosiphon stamineus* Benth (misai kucing) on Sprague Dawley rats. *Trop Biomed.* 2008;25(1):9-16.
  136. Abdullah NR, Ismail Z, Ismail Z. Acute toxicity of *Orthosiphon stamineus* Benth standardized extract in sprague dawley rats. *Phytomed.* 2009;16(2-3):222-226. doi:10.1016/j.phymed.2007.04.013
  137. Muhammad H, Sulaiman SA, Ismail Z, Paumgartten FJR. Study on the developmental toxicity of a standardized extract of *Orthosiphon stamineus* in rats. *Brazilian J Pharmacogn.* 2013;23(3):513-520. doi:10.1590/S0102-695X2013005000039