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Review

An Updated Review of Ethnobotany, Ethnopharmacology, Phytochemistry and Pharmacological Activities of *Orthosiphon stamineus* Benth

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ABSTRACT

There are a myriad of potential medicinal plants worldwide. Researchers have found some, while others are still unexplored. *Orthosiphon stamineus* Benth. (family: Lamiaceae), commonly known as the cat's whiskers plant, is a well-known herbal plant with many medicinal uses. All the parts of *O. stamineus*, such as the leaves, stems, flowers, and seeds, have their uses. More than 140 pure compounds were identified and isolated from different extracts of *O. stamineus*, particularly leaf extracts and stem oils. Flavonoids are the most abundant phytoconstituents found in *O. stamineus* leaf extract. Several pharmacological activities have been studied and validated using extracts as well as pure compounds. Multiple phytoconstituents attributed to these pharmacological activities such as diuretics, antihyperglycemic, antioxidant, hepatoprotective, antiepileptic, etc., have been discovered. Considering the purported benefits of *O. stamineus*, the present review critically evaluates and discusses the pharmacognosy, taxonomy, ethnobotany, pharmacological activities, and phytochemistry of *O. stamineus* and provides updated information.

Key words: Biodiversity, drug, herbal medicine, medicine, natural product, species richness

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INTRODUCTION

A wide variety of medicinal plants have been reported in scientific documents, and their pharmacological activities are assessed to prove traditional uses. Already, over 80,000 medicinal plants have been documented and are being used worldwide, of which more than 1300 plant species are used traditionally in Malaysia, where the conventional knowledge is bequeathed to the next generation (Hossain *et al.*, 2014).

Medicinal plants have been used by primitive people and are still being recognized and accepted today for many uses such as treating diabetes, hypertension, fever, and other beneficial uses (Urbi *et al.* 2014; Hossain *et al.* 2021a). Many people still prefer to use traditional plants to cure a particular disease. Moreover, either directly or indirectly, over 75–80% of citizens from developing countries and approximately 25% of citizens of developed countries depend on different types of medicinal plants for their primary treatment (Ismail *et al.*, 2015). The statements in the divine scripts about the benefits of medicinal plants further extended their usability (Hossain *et al.* 2016; Hossain *et al.* 2021b). The documentation of ethnobotany, pharmacognosy, and taxonomy, and the phytoconstituents' pharmacological effects play a major role in producing advanced treatment by using the plants and their species. It has been discovered that all parts of plants have their uses, including the leaves, flowers, stems, and roots. Therefore, it is crucial to discover and understand more about their potential uses, as they can be used to treat various diseases.

Orthosiphon stamineus Benth (family: *Lamiaceae*) is the synonym of *Orthosiphon aristatus* var. *aristatus,* and commonly known as cat's whiskers. The native range of this variety is from Tropical and subtropical Asia to North Australia. It is a subshrub or shrub and grows primarily in Wet Tropical biomes (POWO, 2022. Considering the medicinal importance of *O. stamineus*, it is crucial to evaluate existing scientific reports for its purported benefits (Singh *et al.* 2015). *Orthosiphon stamineus* is the most common species, and diabetes treatment is the most recognized and agreed-upon in society. Primitive people used several of those species. Several studies and researchers have also reported this herbal plant for commercialization worldwide. Therefore, this review critically assesses and summarizes the plant's pharmacognosy, ethnopharmacology, phytochemistry, and pharmacological activities.

***Orthosiphon stamineus* Benth**

Distribution and taxonomy

Orthosiphon stamineus is native to, Bangladesh, Bismarck Archipelago, Borneo, Cambodia, China South-Central, China Southeast, Hainan, India, Jawa, Laos, Lesser Sunda Is., Malaya, Maluku, Myanmar, New Guinea, Philippines, Queensland, Sri Lanka, Sulawesi, Sumatera, Taiwan, Thailand, Vietnam. This plant is distributed to some other countries, including Caroline Is., Fiji, and Niue (POWO, 2022). The taxonomic classification is given in Table 1, and the vernacular name is described in Table 2 (Almatar & Rahmat, 2014).

Table 1. Taxonomic classification of *O. stamineus*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	*Lamiaceae*
Genus	*Orthosiphon*
Species	*Orthosiphon stamineus* Benth

Table 2. The vernacular name of *O. stamineus,* around the world

Language/country/region	Vernacular name
Malay	Misai kucing
Mandarin	mao xu cao
Tamil	Poonai meesai
Indonesia	Kumis kucing, remujung
Thailand	Yaa nuad maew (Central & Northern), Nuad maew tua ma (Loei), Chatra phra inn (Central), Bang rak paa (Prachuap khiri khan)
Javanese	Remuk jung
France	Moustaches de chat
Europe	Java tea
Burmese	Kant gyok
Cambodian	Kapen prey, mo ni mer cla.
Laotian	Ya kwang, nouat meo
Vietnamese	Hobau kodreng koweng, cay E muoi, cay E nui, nao, rau meo

Plant description and ethnobotany

Orthosiphon stamineus is an herb of perennial origin with a height of approximately one meter. This species needs full sunlight and moist garden soil to grow well. An article stated that this plant grows perfectly under light shade but not in overly shady and warm climates (Adnyana *et al.*, 2013). This plant has green leaves and white flowers (Figure 1).

Stem

These two herbal plants produced a fibrous root structure (Singh *et al.*, 2015). The stem height is approximately not more than 30 cm. The stem is quadrangular, erect, profusely branching and reddish-coloured. They have stamens of more than 2 cm long and ovate leaves with serrated margins extending from the corolla tube—racemes of flowers at the end of branches. The stamens and pistils extend far beyond the petals as the flowers open. In addition, purple and white varieties of *O. stamineus* plant possess the same colour stem. The difference between these two varieties is on the external stem morphology, the white variety had a green colour stem while the purple variety possessed maroon mixed with green colour. Another difference is observed in the internode of both varieties, the purple variety shows a longer internode than the white variety.

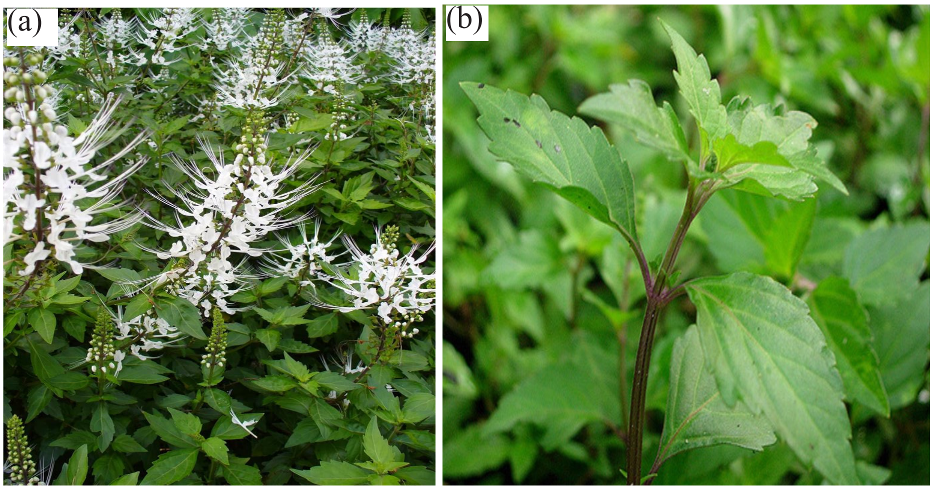


Fig. 1. *Orthosiphon stamineus* flowers (a) and leaves (b)

Flowers

The flowers (Figure 2) are typically white but can be found in luminous shades of purple or violet (Singh et al., 2015). In Malaysia, there are two varieties of *O. stamineus* plants. They can be distinguished by observing the floral colour, which is difficult to distinguish if not carefully detected. White flowers are one of its varieties, while purplish flowers are another. The purplish colour is not completely dark purple, but it does exhibit light purple, which appears very soft and shining, especially in the sunlight.

Furthermore, the purple variety is believed to possess greater bioactive compounds than the white variety (Lee *et al.*, 2015). Subsequently, researchers also found that the flowers on the lower part grow bigger and bloom rather than on the upper part, showing the uniqueness of the flowers that resemble Christmas trees (Almatar & Rahmat, 2014). In the family of Labiatae, one of the main features that classified *O. stamineus* is a group of flowers arranged on a floral axis. This type of flower is composed of a gamopetalous two-lipped (bilabiate) corolla and produces inflorescence-type racemose verticillasters.

In addition, it was reported that small butterflies with unknown species had been reported involved in pollination at the *O. stamineus* (Van der Pijl, 1972). Each flower had an irregular gamosepalous calyx consisting of five sepals, one of which is ovate. It has also been reported that the sepals had an acuminated apex on each side of the large sepal, and the remaining two sepals are linked to each other to produce a long and sharp apex. The part of the stamen in each flower is fastened to the back of the corolla tube. The flowers have a pistil, which is involved in plant reproduction.

However, several differences were found in the floral structures in terms of colour and size. The noticeable difference that can be observed is the colour of its corolla. Researchers also stated that they named different varieties based on the colour of the corolla. The white corolla of the purple species gave rise to pale purple colour, while the white species' petals were white without a purple tint at the edge. It was believed that the purple tone might be due to anthocyanins present in the plant cell (Stern, 2000). This statement is supported by research and findings, which confirmed in *Orchidaceae* that the purple hue occurred because of anthocyanins (Tatsuzawa *et al.*, 2010). Both varieties displayed different colours of the calyx. Maroon colour calyx was observed in the purple species whereas the white species produces a green colour calyx.

Leaves

A study of the morphology of these two varieties (purple and white varieties) was conducted in

Penang, Malaysia, showing dark-coloured green simple leaves, arranged oppositely. To differentiate these two varieties, there is a slight difference, the purple variety has oval-shaped leaves and scattered light yellowish spots on the leaf surfaces. The white variety has obovate-shaped leaves without any coloured spots. Study on the powder form of plants of both varieties shows the presence of sinuous anticlinal cell wall of the leaf epidermis, diacytic stomata, simple uniseriate trichomes, fibers, tracheids, and fragments of spiral thickening vessels. Other studies were also been carried out such as colour tests, purity tests, and so on (Arafat *et al.*, 2008).

Fruits and Seeds

Seed morphology has also been considered one of the significant plant identification characteristics. That is due to the apparent features of the surface of the seeds. These two varieties have ovoid-shaped seeds, and the outer layer of the seeds is irregular. To compare these two varieties, the colour of the full-grown fruits can be differentiated. The purple species may be said to have formed purplish red fruits, while the white species fruit was a combination of green and red colour (Gunn, 1972).

Ethnopharmacology

Orthosiphon staminues is renowned among older people for its medicinal uses. It can be found in many countries and regions, such as Malaysia, Indonesia and Southeast Asia. This plant was first discovered to treat kidney stones, diabetes and hypertension (Tezuka *et al*., 2000). Another famous species in South East Asia and India, *O. pallidus* Royle ex Benth, is also recognized for having many medicinal benefits, including for treatment of oedema, urinary lithiasis, influenza, fever, jaundice, hepatitis and rheumatism (Singh *et al.*, 2017). Another species found in India is *O. thymiflorus*, which is used to treat cytotoxicity, diabetes, inflammation and hypertension (Sundarammal *et al.*, 2012). The leaf extract of *O. stamineus* is used as a diuretic by the Southeast Asian community (Gimbun *et al*. 2019). Decoctions of *O. stamineus* have been used in folk medicine to treat various kidney diseases such as renal calculi and urinary tract infection (UTI). A decoction of dried leaves is often used to treat strangury and dysuria (Gimbun *et al*. 2019). According to a study, traditional medical practitioners used dried or fresh whole plant parts to flush out kidney stones (Muhlisah, 2007). Filipinos used the decoction of the leaves to relieve gout (De Padua *et al.*, 1987). Another study showed that the Kenyah people, who originated from Sarawak, Malaysia, used the young twigs and leaves of *O. stamineus* to treat back pain (Chai, 2006). In Thailand, the leaves are pounded up, warmed over a fire while wrapped in bamboo leaves, and put on bruised or sprained ankles (POWO, 2022).

Phytochemistry

Extensive phytochemical analyses of *O. stamineus* extracts have been conducted by researchers since the 1930s. Until now, more than 100 chemical compounds have been identified by scientists. These compounds have been classified as flavonoids, terpenoids (i.e., monoterpenes, diterpenes, triterpenes), saponins, organic acids, and essential oils. More than a decade ago, Hossain *et al.* (2008) conducted an extensive study in Malaysia and reported 69 phytocompounds of *O. stamineus* isolated from whole leaf methanol extracts and stem oil, which were fractionated using different organic solvents, namely hexane, chloroform and ethyl acetate. Among the identified compounds, the major compounds were β-caryophyllene, α-humulene, β-elemene, 1-octen-3-ol, β-bourbonene, β-pinene, caryophyllene oxide, camphene and limonene. However, the yield varied significantly depending on the source of the extracts. For example, β-caryophyllene was found at 24% in the leaf extract, whereas it was 35% in stem oils. It is noted that some compounds, such as β-bourbonene (3.4 vs. 3.0%), β-pinene (2.1 vs. 1.7%), caryophyllene oxide (1.6 vs. 2.2%), camphene (1.6 vs. 1.3%), and limonene (1.2 vs. 1.1%), were present in an almost equal amount regardless of the type of samples (leaf extract vs. stem oils). This study reported a great potential of antifungal activity against different pathogenic species: *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium solani*, *Colletotricum capsici* and *Phytophthora capsici*. This finding suggested that the antifungal activity might be attributed to the presence of major compounds, including essential oils, β-caryophyllene, caryophyllene oxide, α-humulene, β-pinene, limonene, β-elemene, and 1-octen-3-ol in the extracts of *O. stamineus*, as these compounds' antifungal activity have been reported previously by many scientists (Costa *et al.*, 2000; Filipowicz *et al.*, 2003; Hammer *et al.*, 2003; Okull *et al.*, 2003; Sacchetti *et al.*, 2004; Deba *et al.*, 2008). A few years ago, more than 140 compounds of *O. stamineus* were compiled from different scientific reports (Ameer *et al.*, 2012).

In a recent study by Joshi (2020), the hydro-distilled essential oil from aerial parts of *O. pallidus* Royle, ex Benth (Lamiaceae) was investigated using gas chromatography equipped with a flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). Approximately 52 compounds were identified, accounting for almost 100% of the total oil constituents. The main constituents were *β*-caryophyllene and 7-*epi*-*α*-selinene. The remaining minor constituents were terpinolene, *β*-pinene, *β*-elemene, *α*-humulene, *α*-copaene, *epi*-cubebol and zonarene. Zonarene was the least extracted constituent in this experiment.

Some other phytochemical studies also indicated that the plant *O. stamineus* contains phytochemicals such as orthocromene A, acetovanillochromene (Shibuya *et al.*, 1999), methylripariochromene A (Guerin *et al.*, 1989), orthosiphols A and B (Masuda *et al.*, 1992), orthosiphols F,G,H and I, staminol (Stampoulis *et al.*, 1999), pimarane-type diterpenes (neoorthosiphols A and B) (Shibuya *et al.*, 2010), and essential oil 0.02-0.7% which consist of various compounds including β-elemene, β-caryophyllene, α-humulene, β-caryophyllene oxide, can-2-one and palmitic acid (Schut & Zwaving, 1986). Flavonoids including eupatorine, sinensetin (Laavola *et al.*, 2012), tetramethylscutellarein and other tetramethoxyflavones, salvigenin, cirsimaritin, pilloin, rhamnazin, trimethylapigenin and tetramethylluteolin (Wollenweber & Mann, 1985; Malterud *et al.*, 1989), caffeic acid, rosmarinic acid, inositol and phytosterols (e.g. β-sitosterol) (Sumaryono *et al.*, 1991; Lyckander & Malterud, 1996) were also discovered. Table 3 presents the phytochemistry of these compounds.

PHARMACOLOGICAL ACTIVITIES

Diuretic activity

The diuretic activity, elucidating its viable mechanism as well as evaluating the renal outcomes of Orthosiphon stamineus extract, was reported by Adam *et al.* (2009). The extracts of *O. stamineus* functioned in a dose-dependent manner in diuretics. In rats, a study of the *O. stamineus* leaf extracts of methanol demonstrated its diuretic and hypouricemic effects (Arafat *et al.*, 2008). This study used a positive control of hydrochlorothiazide (10 mg/kg). For seven consecutive days, extracts of methanol (methanol and water ratio: 1:1) were given orally at 0.5 g/kg. Cumulative urine concentrations and electrolytes, such as sodium and potassium, were measured at different intervals. These findings showed a strong propensity for diuretic and hypouricemic effects in rats.

Antiurolithiatic/ Nephroprotective activity

The nephroprotective effect of *O. stamineus* Benth was confirmed by reducing the creatinine and urea concentration, dropping the level of proteinuria, and increasing the glomerular filtration rate (GFR) and diuresis compared to controls (Mannavalan, 2010). Another study explained how O. stamineus has been traditionally used to treat kidney stones by removing the binding of the energetic compounds to adenosine A1 receptors. This lets them connect to diuretic activity, which could be used to treat renal lithiasis (Yuliana et al., 2009).

Another study discovered the *in vitro* antiurolithiatic effects of *O. stamineus* decoction (Wan Marzuki Rashidi *et al*. 2019). In addition, in the same study, a nucleation assay was carried out to discover the inhibitory effects in the direction of calcium oxalate crystallization urolithiasis *in vitro*. *Orthosiphon stamineus* extract exhibited the highest inhibition rate (73.48%) against calcium oxalate, which can be attributed to its saponins, tannins, steroid and terpenoid content. In a study by (Ramesh *et al*., 2014), the ethanolic extracts of *O. stamineus* were investigated for nephroprotective activity in albino rats. Thylene glycol was used to induce urolithiasis. The results showed that plant ethanolic extracts validated nephroprotective activity compared to standard drugs.

Antihyperglycemic activity

Orthosiphon stamineus is well known for its benefit, which helps lower the blood sugar level in diabetes mellitus (DM) patients. A study validated the use of this extract for DM in Streptozotocin (STZ)-induced rats with the dose of 500 mg/kg body weight (BW), p.o. for two weeks (Azam *et al.* 2017). Mohamed *et al.* (2011) studied the extracts of *O. stamineus* leaves obtained using four solvents to evaluate antihyperglycemic effects. Among the four extracts, the chloroform extract showed a remarkable reduction in the glucose level at a dose of 1g/kg BW in the rat model, injected subcutaneously with glucose of 150 mg/kg BW.

Another study demonstrated the hypoglycemic effects of *O. stamineus* extract in pregnant and non-pregnant STZ-induced diabetic rats. An oral glucose tolerance test was performed in pregnant and non-pregnant rats before and after treatment with *O. stamineus* (0.1 g/100 g of BW). *O. stamineus* extract was given orally every day for fourteen days to non-pregnant rats and ten days to pregnant rats. An oral glucose tolerance test indicated that treatment with *O. stamineus* in non-pregnant and pregnant rats significantly reduced blood glucose levels and stimulated glucose-induced insulin secretion (Lokman *et*

Table 3. Phytochemistry details of compounds

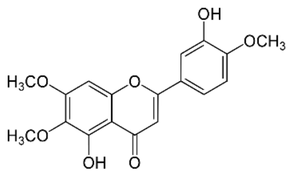
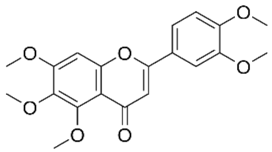
Compound	Structure	Class	Example of sources	References
Eupatorin			• Isolated from chloroform extract of *O. stamineus*	(Laavola *et al*., 2012)
			• Isolated from chloroform extract of *O. stamineus* leaves	(Dolečková *et al*., 2012)
			• Validated by a simple isocratic HPLC Method in different types of *O. stamineus* extracts and fractions	(Yam *et al*., 2012)
			• Isolated from ethanolic extract of *O. stamineus* leaves	(Shafaei *et al*., 2016)
			• Validated by high-performance liquid chromatography (HPLC) method	(Saidan, 2015)
Sinensetin		Flavonoid	• Isolated from chloroform extract of *O. stamineus* leaves	(Laavola *et al*., 2012)
			• Validated by a simple isocratic HPLC Method in different types of *O. stamineus* extracts and fractions	(Yam *et al*., 2012)
			• Validated by high-performance liquid chromatography (HPLC) Method of *O. stamineus*	(Saidan, 2015)
			• Isolated from ethanolic extract of *O. stamineus* leaves	(Shafaei *et al*., 2016)
			• Validated by using Ultra HPLC coupled with electrospray ionization tandem mass spectrometry of *O. stamineus*	(Guo *et al*., 2019)
			• Isolated from the mixture solvent of acetone-water of *O. stamineus* leaves	(Amzad Hossain & Ismail, 2016)

Table 3. *Continued*

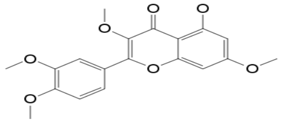
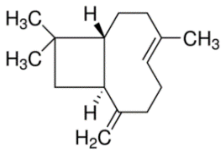
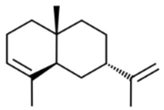
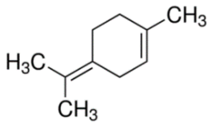
Compound	Structure	Class	Examples of sources	References
3-hydroxy-5, 6, 7, 4-tetramethoxy flavone			• Validated by a simple isocratic HPLC Method in different types of *O. stamineus* extracts and fractions	(Yam *et al*., 2012)
			• Validated by high-performance liquid chromatography (HPLC) method of *O. stamineus*	(Saidan, 2015)
Major constituents:			• Analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) of leaves of *O. stamineus*	(Azizan *et al*., 2017)
β-caryophyllene			• Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification	(Joshi, 2020)
			• Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification	(Joshi, 2020)
			• Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification	(Joshi, 2020)
7-*epi*-*α*-selinene		Sesquiterpene		
Minor constituents:				
Terpinolene				

Table 3. *Continued*

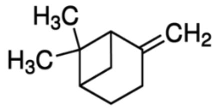
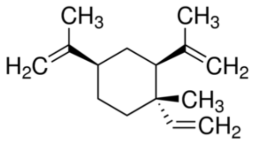
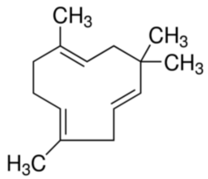
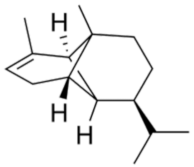
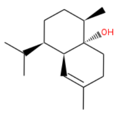
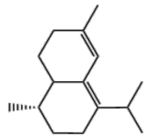
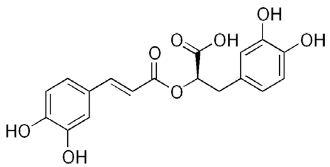
Compound	Structure	Class	Examples of sources	References
β-pinene			• Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification • Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification	(Joshi, 2020) (Joshi, 2020)
β-elemene			• Analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) of leaves of *O. stamineus* • Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification	(Azizan *et al*., 2017) (Joshi, 2020)
α-humulene		Sesquiterpene	• Analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) of leaves of *O. stamineus* • Analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) of leaves of *O. stamineus*	(Azizan *et al*., 2017) (Azizan *et al*., 2017)
α-copaene				
epi-cubebol				

Table 3. *Continued*

Compound	Structure	Class	Examples of sources	References
Zonarene		Sesquiterpene	• Isolated by gas chromatography equipped with a GC-FID and GC-MS from Orthosiphon same family classification	(Joshi, 2020)
Rosmarinic acid		Polyphenol	• Isolated from the mixture of chloroform-ethyl acetate of *O. stamineus* • Isolated and analyzed by High Performance Liquid Chromatography (HPLC) of *O. stamineus* • Extracted by reflux reactor of leaves and stems *O. aristatus*	(Lau & Chua, 2019) (Zhang *et al*., 2019) (Chua, Lau, Chew, Ismail, & Soontorngun, 2018)

al., 2019). Another study conducted by Xu *et al.* (2019) emphasized the usage of *O. stamineus* in type 2 DM and showed a positive result. It concluded that there was no toxicity and that it is safe for use in gestational diabetes.

In one study, researchers evaluated the ability of *O. stamineus* extract in insulin-producing cells to prevent glucotoxicity (Lee *et al.*, 2015). After exposure to a high glucose concentration, they measured insulin mRNA expression and glucose-stimulated insulin secretion (GSIS) in INS-1 cells treated with *O. stamineus*. *O. stamineus* hexane extract raised the dose-dependent expression of mRNA in insulin and pancreatic and duodenal homeobox-1 of INS-1 cells. The treatment with a particular amount of hexane *O. stamineus* extract also increased Akt phosphorylation. When three days of hyperglycemia exposure suppressed the expression of insulin mRNA and GSIS, *O. stamineus* extracts restored the expression of mRNA and normal blood glucose level. In this stage, hyperglycemia elevated levels of peroxide in cells with INS-1 under both normal and hyperglycemic conditions. These findings suggested that *O. stamineus* hexane extract elevates the expression of insulin mRNA and prevents glucotoxicity induced by hyperglycemia for three days. This has been linked with the activation of PI-3K and Akt (Lee *et al.*, 2015).

Antioxidant activity

Antioxidant activity of *O. stamineus* extract was assessed by *in vitro* DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Akowuah *et al.*, 2005). The result displayed that the acetone extract exhibited significant antioxidant activity (Akowuah *et al.*, 2005). Another study evaluated antioxidant activity by Folin-Ciocalteu, aluminium trichloride, β-carotene bleaching and DPPH assays. As a result, *O. stamineus* reduced the β-carotene oxidation by hydroperoxides (Abdelwahab *et al.*, 2011). Antioxidant and antibacterial activities of leaf extracts of *O. stamineus* and *Andrographis paniculate* were evaluated (Malahubban *et al.*, 2013). Both plant extracts displayed a powerful antioxidant effect. Antioxidant activity and intestinal protective effect of the root, stem and leaves of *O. stamineus* extracts were reported by Cai *et al.* (2018). The results showed that root extracts of *O. stamineus* had the highest amount of polyphenol and flavonoid constituents as well as the highest free radical scavenging activity.

Hepatoprotective activity

Hepatoprotective activity was exhibited by *O. stamineus* in Carbon tetrachloride-induced hepatotoxic rats (Yam *et al.*, 2009). The hepatoprotective activity may be due to the existence of antioxidants and scavengers of free radicals in mammalian cells. The experiment was conducted for three days, and selective doses, such as 500 and 1250 mg/kg BW, showed the greatest bilirubin reduction. The active methanol fraction of *O. diffuses* was tested *in vivo* and *in vitro*. Compared to silymarin, the fraction exhibited a hepatoprotective effect (Ghaffari *et al.*, 2013).

Antipyretic activity

A study reported the antipyretic activity of *O. stamineus* extract in rats (Yam *et al.*, 2009). Selected doses of 500 and 1000 mg/kg BW substantially decreased the body temperature induced by the pyrogen. The duration of action lasted about four hours after administration. And it performed well comparable with the standard drug, paracetamol, 150 mg/kg BW.

Antihypertensive activity

Another study revealed the antihypertensive activity of a component, methyl ripario chromene A (MRC) isolated from the leaves of *O. stamineus*. MRC was administered to the experimental rats subcutaneously (Matsubara *et al.*, 1999). Lowered systolic blood pressure, as well as heart rate, were observed in the experimental rats after administration of MRC (Matsubara *et al.*, 1999). Another study reported that blood pressure reduction could be observed in hypertensive rats treated with an extract of *O. stamineus* leaves (Nurul Alia *et al.*, 2012). The extract produced an angiotensin-converting enzyme (ACE) inhibitory action (Shafaei *et al.*, 2016).

Previous studies have shown that the content of 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF) in fractions of *O. stamineus* correlates with its vasorelaxant activity (Tan & Yam, 2018). The vasorelaxation activity and the underlying mechanisms of TMF were evaluated on thoracic aortic rings isolated from Sprague Dawley rats. The present study shows that TMF's vasorelaxant effect involves nitric oxide pathways, soluble guanylate cyclase, cyclic guanosine monophosphate and prostacyclins, calcium and potassium channels, and muscarinic and beta-adrenergic receptors (Tan & Yam, 2018).

Antimicrobial activity

Tong *et al.* (2011) investigated various endophytic fungi isolated from different parts of *O. stamineus*. most fungi were isolated ~~was~~ from the leaves of the plant. It was found that the methanolic extract exhibited remarkable antimicrobial activity compared to ethyl acetate extracts. The culture medium affected the isolated fungi, and the antimicrobial activity increased with the addition of water extract from a particular host plant. *O. stamineus* is well known for its higher rosmarinic acid content and is reported to have the best antibacterial effect (Ho *et al.*, 2010). The bacteria used in this experiment was *Vibrio parahaemolyticus*, a Gram-negative bacterium that may cause gastrointestinal illness in humans if ingested. Furthermore, a study implementing the disc diffusion method and minimum inhibitory concentration concept demonstrated the best antimicrobial activity against *Staphylococcus aureus* (Alshawsh *et al.*, 2012).

Antiulcer activity

Antiulcer activity of the *O. stamineus* leaf extract was examined in experimental rats injected with aspirin (Yuniarto, 2017). The study showed potential gastric ulcer healing activity exhibited by the *O. stamineus* leaf extract. The antiulcer activity was evaluated by measuring several parameters such as gastric acidity, number of ulcers, ulcer diameters, ulcer index (UI) and healing ratio. Sucralfate 90 mg/kg was used as a standard drug. *O. stamineus* extract at a dose of 250 mg/kg BW and 500 mg/kg BW effectively impacted the stated parameters. This result is consistent with the histopathological examination, where the stomach tissue and inflammation area were reduced compared with the control group (aspirin 500 mg/kg BW) in groups given treatments with leaf extracts (Yuniarto, 2017).

In another study, the antiulcer activity of *O. stamineus* leaf extract was evaluated against ulcers induced by ethanol in rats. *O. stamineus* methanolic extract was administered orally in doses of 125, 250, 500, and 1,000 mg/kg BW and was found to decrease the ulcer index significantly (Yam *et al.*, 2009). The histological study of the rat stomach section observed a marked improvement in the damage to gastric mucosal. The mucus secretion and the level of lipid peroxidation were estimated *in vitro* and *ex vivo* for further investigation of the gastroprotective mechanism of the methanolic extract. The methanolic extract demonstrated dose-dependent stimulation of mucus secretion in rat gastric mucosal homogenates and suppression of lipid peroxidation (Yam *et al.*, 2009).

Antiepileptic activity

The antiepileptic effect of ethanol extract of *O. stamineus* leaf on a zebrafish model was investigated. The leaf extract's showed anti-convulsive activity and ~~is~~ also comparable with that of diazepam (Choo *et al.*, 2018). After a seizure with pentylenetetrazol (PTZ), extract therapy was also shown to counteract the upregulation of Nuclear Transcription Factor Y Subunit Beta, Neuropeptide Y and Tumor Necrosis Factor Alpha (Choo *et al.*, 2018). Since the ethanolic extract *O. stamineus* possesses the highest amount of phenolic compounds it showed significant antioxidant activity that leads to remedies for epilepsy utilizing the plant leaves. Additionally, it is noted that this plant is traditionally used for epilepsy treatment (Saidan *et al.*, 2015), (Hossain & Rahman, 2015).

Immunomodulatory activity

The immunostimulating effect of *A. muricate, O. aristatus* on phagocytic activity of leukocytes was investigated and the effect could be due to the presence of active compounds in these plants, which triggers the complementary pathway and generates C3b and C3bi, which bind to *E. coli*. The~~-~~bounded *E. coli* was recognized by phagocyte receptors such as CR1 (CD35) and CR3 (CD11b) and begin phagocytosis. The phagocytosis process is built up by the expression of Fcγ receptors such as FcγRIII (CD16) and FcγRII (CD32). The stimulation effect is caused by the direct influence of active compounds present in these plants, which increases the expression of Fcγ receptor on the phagocyte cells and then stimulates opsonized *E. coli* binding on a receiver (Harun *et al.*, 2015).

Anti-inflammatory activity

One study demonstrated the anti-inflammatory activity of leaf extracts of *O. stamineus* and identified active compounds that contribute to thisactivity (Yam *et al.*, 2010). The three fractions of an active chloroform extract of *O. stamineus* were investigated using the carrageenan-induced hind paw oedema method to inhibit anti-peritoneal capillary permeability, *in vitro* nitric oxide scavenging activity, and anti-inflammatory activity. The fraction of flavonoid-rich chloroform extract significantly reduced rat hind paw oedema and nitric oxide and decreased peritoneal cavity dye leakage. The fraction of flavonoid-rich chloroform extract scavenging IC50 was 0.3 mg/mL *in vitro* nitric oxide (Yam *et al.*, 2010). These results suggest that these chloroform extracts' anti-inflammatory properties may be due to the presence of flavonoid compounds capable of affecting the nitric oxide pathway.

Anti-arthritic activity

Orthosiphon stamineus ethanol (50%) extract possessed an *in vitro* anti-arthritic effect in Freund's complete adjuvant (FCA) induced arthritis model using an *in vitro* heat denaturation test. A significant inhibition in the production of tumour necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), COX-1, and COX-2 was observed in the experimental rats treated with the extract. The X-ray result showed an improvement in the joint condition of the back limb of a quadruped. Progress could also be observed in the bones of the tibia and talus of the foot. Histology results indicated a visible part of cartilage and soft tissues surrounding it were protected by the effect of *O. stamineus* extract. Hence, it was found that *O. stamineus* extract exhibited anti-arthritic activity (Tabana *et al.*, 2016).

Anti-obesity activity

Seyedan *et al.* (2016) reported the anti-obesity effect of an ethanolic extract of *O. stamineus* leaf (200 and 400 mg/kg) and its primary compound (rosmarinic acid, 10 mg/kg) in overweight mice (C57BL/6) triggered by a high-fat diet. Ongoing supplementation with *O. stamineus* extract (200 and 400 mg/kg) for eight weeks significantly reduced the BW. Compared with the high-fat diet-fed group, serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels within the treated groups were significantly reduced. In contrast, the high-density lipoprotein cholesterol levels were not altered considerably. *Orthosiphon stamineus* extract markedly inhibited the accumulation of hepatic lipid droplets triggered by using a high-fat weight loss plan. Additionally, *O. stamineus* drastically reduced the production of liver malondialdehyde and substantially increased the hepatic superoxidase dismutase activity (Seyedan *et al.*, 2016). So, it can significantly reduce BW benefits, increase antioxidant activity and have its own hypolipidemic and anti-obesity outcomes.

Another study hypothesized that in high-fat-fed obese animals, a combination treatment of leptin sensitizer and endogenous leptin expression stimulant would synergistically induce an anti-obesity effect (Choi *et al.*, 2013). A common feature of obesity is leptin resistance which is accompanied by hyperleptinemia. Although leptin sensitizers improve leptin resistance, they also decrease levels of plasma leptin, which attenuates the anti-obesity effect associated with leptin. To assess the hypothesis, researchers treated obese mice for 14 days with betulinic acid and the herbal plant extract. The experimental animals were fed a high-fat diet for six weeks (Choi *et al.*, 2013). *The O. stamineus* ethanol extract induced dose-dependent leptin expressions in the 3T3-L1 adipocytes and mice. Although betulinic acid and *O. stamineus* alone did not decrease BW in obese mice, the combinational treatment of the acid and the plant extract significantly decreased BW compared with either betulinic acid or *O. stamineus* treated obese mice. These results suggest that combinatorial therapy would be effective in treating obesity.

Anti-tumour activity

In one study, standardized 50% ethanol extract of *O. stamineus* reported antitumor potential *O. stamineus* was assessed against colorectal tumours in athymic mice, and the antiangiogenic efficacy of the herbal plant was investigated in the endothelial human umbilical vein. The higher the extract dose, the greater the tumor growth suppression. In addition, *O. stamineus* abolished the function of vascular endothelial growth factor (VEGF), which acts like other anti-VEGF drugs (Ahamed *et al.*, 2012).

Cytoprotective, antimutagenic and anticlastogenic activities

In another study, *O. stamineus* ethanolic leaf extract was reported to protect lining tissues from cell damage, prevent mutagenicity and inhibit damage to the chromosomes. Hydrogen peroxide and serum-deprived medium were used to expose the normal liver cell lines. The results revealed that *O. stamineus* extract was a potent scavenger in the serum-deprived medium for hydrogen peroxide and other free radicals. This research revealed the potential of this herbal plant extract for cytoprotective, antimutagenic and anticlastogenic activities (Al-Dualimi *et al.*, 2018).

TOXICITY

A study demonstrated possible toxic effects after two weeks of oral administration of methanol extract of *O. stamineus* in female Sprague Dawley rats. However, this experiment showed no toxicity exhibited by methanol extract of *O. stamineus* within the study duration (Chin *et al.*, 2008). It can be concluded that

O. stamineus extract is safe, and there is no organ damage in the model rats by the incident of lethality and blood serum biochemical parameters (Chin *et al.*, 2008). Another study was conducted to assess the safety of the *O. stamineus* plant's standardized 50% ethanol extract by determining its potential toxicity after acute and subchronic administration in rats for 14 days and 28 days of study respectively (Mohamed *et al.*, 2011). The rat model was given specific doses, and the study's outcome exhibited no toxicity in the rats. A similar report was also reported by Muhammad *et al.* (2013) and stated that methanol extract of *O. stamineus* within the range of 0.5 g/kg, 1 g/kg, 3 g/kg and 5 g/kg BW would not cause any severe toxic effects and organ damages in rats.

Another study assessed the developmental toxicity of standardized aqueous extract of *O. stamineus* in female Sprague Dawley rats from low to high doses (250, 500, 1000 and 2000 mg/kg/day) by gavage on gestation days six to twenty. During the study, maternal toxicity, body weight, and food/water consumption were monitored. On gestation day 21, O. stamineus extract exhibited no negative impact on maternal factors. Nevertheless, it caused an increase in skeletal variations and skull bone malformations in fetuses in a non-dependent manner. In a non-dose-dependent manner, In male and female fetuses exposed to the highest standardized aqueous extract dose of *O. stamineus*, Tthe increased distance from the midpoint of the anus to the genitalia was observed in the fetuses that exposed to the highest dose of extract. Researchers assumed that this herbal extract could possibly contain androgenic compounds (Muhammad *et al.*, 2013).

Dried leaves of *O. stamineus* were used to evaluate the possible toxicity by Pariyani *et al.* (2015). The acute oral toxicity test was assessed in Sprague Dawley rats using aqueous, 50% aqueous ethanolic, and ethanolic extracts of *O. stamineus* at a dose of 5000 mg/kg BW. The animals were observed during a fourteen-day study for any mortality, reactions toward the extract, motor-neuronal defects, BW, and feed-water consumption pattern. In addition to the histological analysis of the kidney and liver, haematological and serum biochemical parameters were checked to assess the kidney and liver functions. The dried leaves of *O. stamineus* showed no toxicity or mortality for the administered dose. Hence, researchers stated that dried leaves of this herbal plant had the potential oral lethal dose of more than 5000 mg/kg of BW.

Genotoxicity

A study explored the genotoxic effect of *Salmonella* using the microsome mutation assay and the micronucleus test of the mouse bone marrow (Muhammad *et al.*, 2011). The *O. stamineus* extract does not give any genotoxicity towards *Salmonella* tested strains. In the mouse bone marrow assay, the extract did not alter the polychromatic state, which indicated that the *O. stamineus* extract had no genotoxic risk. Another genotoxicity study revealed *O. stamineus* contained a variety of compounds which may help protect against cellular damage caused by mutagens (Al-Dualimi *et al.*, 2018). The herb has shown anti-oxidative properties and can modulate key cellular proteins that have a cytoprotective effect. Treatment of this herbal extract at different doses set up before and after mitomycin c (MMC) injection has been shown to have several parameters related to genotoxicity (Muhammad *et al.*, 2011; Al-Dualimi *et al.*, 2018). The study suggested this plant extract's renoprotective potential.

CONCLUSION

The taxonomy, pharmacognosy, ethnobotany, pharmacological activities and phytochemistry of O. stamineus have been reviewed and discussed in this paper. Many studies have validated the use of *O. stamineus* in treating various diseases. As this traditional herbal plant is available easily and widely used worldwide nowadays, more studies are necessary to refine the use of this plant. This review explores the benefits of *O. stamineus* plant and will be helpful for future research to carry out experiments based on *O. stamineus*.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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