Orthosiphon stamineus (Lamiaceae) is a valued medicinal plant in traditional folk medicine. Many pharmacological studies have demonstrated the ability of this plant to exhibit antimicrobial, antioxidant, hepatoprotection, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, antidiabetic, anti-inflammatory activities. This review is a comprehensive summary of the presently available chemical, pharmacological investigations as well as the traditional and therapeutic uses of this plant. Important and different experimental data have been addressed along with a review of all phytochemicals identified in this plant, including flavonoids, terpenoids, and essential oils. *O. stamineus* has wide traditional and pharmacological uses in various pathophysiological conditions. Therefore, it is an attractive subject for further experimental and clinical investigations.

**KEYWORDS:** Misai kucing, Orthosiphon stamineus, rosmarinic acid, sinensetin
Phytochemical Study of *O. stamineus*

The phytochemical study of *kumis kucing* grown in Asia has been conducted extensively since the 1930s. The scientists have identified more than hundreds of chemical compounds and classified them as monoterpenes, diterpenes, triterpenes, saponins, flavonoids, organic acids, and so on. Moreover, earlier study has recognized 69 chemical compounds in the essential oil extracted from the leaves of *O. stamineus*. They were 1-octen-3-ol, β-bourbene, β-caryophyllene, α-humulene, β-elemene, phenylacetaldehyde, caryophyllene oxide, β-pinene, camphene, 3-octanol, limonene, cis-2-octenal, 2-heptenal, trans, cis-octa-3-5-dien-2-one, 1-methylnaphthalene, α-munielone, trans, trans-octa-3-5-dien-2-one, 2-amylfuran, menthone, carvone, methylevichicol, α-pinene, tridecane, p-cymene, pentenylnifuran, hexanal, naphthalene, benzaldehyde, eugenol, linalool, trans-linalool oxide, δ-cadinene, trans-2-(cis)-6-nonadienale, methyl eugenol, trans-2-hexanal, camphor, citronellole, α-copaene, borneol, dodecane, α-cubebene, geranylacetone, δ-terpineol, acetothenone, trans-anethole, germacrene D, decanal, δ-elemene, 1,8-cineole, 4-heptenal, isomenthone, β-cyclocitril, damascenone, dehydroionone, cis-linalool oxide, undecane, bornyl acetate, 2-methyl naphthalene, β-ionone, perillen, safranal, hexahydrofamesylacetone, hexan-1-ol, 2,6,6-trimethyl-2-cyclohexene-1,4-dione, isobornyl acetate, trans, trans-deca-2,4-dienal, cis-caryophyllene, germacrene, and cis-3-hexen-1-ol.[22] Few years ago, seven triterpenes—namely, ursolic acid, oeleanolic acid, betulinic acid, hydroxyl betulinic acid, maslinic acid, a-amyrin, and b-amyrin—have been isolated from the leaves of *O. stamineus*. Recently, one compound a-amyrin was isolated for the first time from this plant. Some other compounds detected were b-caryophyllene, a-humulene, b-elemene, 1-octen-3-ol, b-bourbonene, b-pinene, caryophyllene oxide, camphene, and limonene. These are all major compounds obtained from the hydro distilled essential oils of the leaves and stems of *O. stamineus*. Alternatively, a-pinene, 1,8-cineole, borneol, linalool, camphor, eugenol, p-cymene, carvone, bornyl acetate, and d-cadinene were reported as minor components of *O. stamineus* leaf and stem oils.[33] Some of the structures of chemical constituents of *O. stamineus* are mentioned in Figure 2. In recent days, several analytical methods were used for the analysis of bioactive constituents found in *O. stamineus*. Saidan et al.[34] developed and validated a novel reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of four marker compounds—RA, 3'-hydroxy-5,6,7,4'-TMF, SIN, and EUP—in numerous *O. stamineus* leaf extracts using RP-HPLC-diode-array detection at 320 nm using a gradient mobile phase of 0.1% formic acid:acetonitrile at a flow rate of 1 mL/min on reverse-phase acclain polar advantage II C18 column (3 μm, 3 × 150 mm) with 18 min separation time. Recently, Hashim et al.[35] developed and validated high-performance thin-layer chromatography method and simultaneously quantified four compounds—RA, 3'-hydroxy-5,6,7,4'-TMF, SIN, and EUP—found in ethanol, 50% ethanol, and water.
In recent times, See Tiam et al.\textsuperscript{36} showed the impacts of various methods such as mechanical grinding, ultrasonic-assisted extraction (UAE), microwave-assisted extraction, and also sample pretreatments using acid and alkali on the microstructure of plant sample for the extraction of

extract of \textit{O. stamineus} leaves. The linearity of RA, TMF, SIN, and EUP were obtained between 10 and 100 ng/spot with high correlation coefficient value ($R^2$) of more than 0.986. The limit of detection was found to be 122.47 ± 3.95 (RA), 43.38 ± 0.79 (SIN), 17.26 ± 1.16 (TMF), and 46.80 ± 1.33 ng/spot (EUP).
bioactive compounds from *O. stamineus* leaf. The results exposed good diffusion of bioactive compound of approximately 86%–95% of the total extraction yield quantified by conventional Soxhlet extraction method. Chemical pretreatments normally reported weaker microstructure disruption; thus, a slight enhancement on the extraction yields was observed. In this case, acid reagent is more appropriate for the pretreatment, as the presence of alkali decomposes the bioactive compounds. In another study, Sree *et al.* developed an HPLC method in which chromatographic separation was carried out using a mobile phase methanol–acetic acid–water (10:2:88, v/v) as solvent A and methanol–acetic acid–water (90:2:8, v/v) as solvent B and programmed in gradient. In another experiment, an RP-HPLC method was developed using an isocratic system with a flow rate of 1 mL/min, a column temperature of 25°C with a mobile phase of acetonitrile:isopropyl alcohol:20 mM phosphate buffer (NaH₂PO₄) (30:15:55, v/v). The UV detection was set at 340 nm. The injection volume was 20 μL of solution with a run time less than 20 min for each injection. The peaks were detected at 340 nm and identified using reference standards.[38]

**Pharmacological Activities**

**Antiproliferative and cytotoxic activities**

*O. stamineus* contains a number of phenolic flavonoid compounds that play a significant role in the treatment of various types of ailments. A study showed that norstaminolactone A, orthosiphols A, B, D, E, K, L, M, N, O, P, and Q, nororthosphonolide A, orthosphonone A, norstaminone A, and neoorthosiph A compounds showed weak-to-mild antiproliferative activity when tested for their cytotoxic activity against highly liver metastatic colon 26-L5 carcinoma and human HT-1080 cell lines. Only norstaminolactone A showed potent antiproliferative activity with inhibitory concentration (IC₅₀) value of 2.16 mg/mL against highly liver metastatic colon 26-L5 carcinoma cell line.[13,24,27,39] Numerous recent studies showed the cellular and molecular mechanism of the antiproliferative activity of *O. stamineus* extracts.[38,40,41] Previous reported works provides better understanding of mechanism of action of anticancer effect of the plant. In another study, Salleh *et al.* showed that ethyl acetate fraction (hot water extract) of *O. stamineus* could prevent the growth of human hepatocellular carcinoma cell line (HepG2) by inducing apoptosis. In this process, nuclear condensation and fragmentation as well as mitochondrial membrane dysfunction (sign of apoptosis) were noticed in the HepG2 cells. The study also suggested that ethyl acetate fraction of *O. stamineus* is a possible candidate for further development of chemopreventive agent for human liver cancer. Other major detected constituents of *O. stamineus* in the ethyl acetate fraction were RA and caffeic acid, and these were suspected to have synergistically backed cancer cell apoptosis. In an in vivo experiment, 50% ethanolic extract of *Orthosiphon* act as anticancer against colorectal tumor in nude mice. In this experiment, orally administered two doses of 100 and 200 mg/kg body weight (BW) of the extract of *O. stamineus* over 4 weeks suppressed tumor growth by 47% and 83%, respectively.[40] Stampoulis *et al.* found that the methanol extract of *O. stamineus* leaves showed a cytotoxic activity against liver metastatic colon 26-L5 carcinoma cells. Remarkably, its chloroform fraction showed the strongest activity. In another study, compounds of *O. stamineus* from Myanmar showed mild-to-weak antiproliferative activity toward highly malignant liver metastatic colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines.[42] Awale *et al.* further studied the possible cytotoxic activity of compounds isolated from Japanese *O. stamineus* toward highly malignant liver metastatic murine colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines. Now day's attention is given in using natural anti-angiogenic compounds instead of synthetic drugs. Sahib *et al.* investigated the effect of methanolic extract of *O. stamineus* and found that this extract can enhance the anticancer efficacy of tamoxifen, an estrogen receptor antagonist. The extract by itself does not exert any appreciable effect. It was also seen that the antiproliferative activity of tamoxifen toward MCF-7 hormone-sensitive breast cancer cells was raised up by fivefold, when it was coadministered with the extract. In general, *O. stamineus* synergistically enhanced the activity of tamoxifen against hormone-responsive breast cancer cells in vitro. Therefore, it is suggested to be useful as an adjuvant for treating metastatic breast cancer. Another research conducted by Abdelwahab *et al.* correlated the antiapoptotic property with antioxidant and phenolic compound content. The antiapoptotic and antioxidant activities of *O. stamineus* aqueous–methanolic extract and its fractions were examined. The results revealed that ethyl acetate fraction contains highest total phenolic content and antioxidant activity, whereas the chloroform fraction was found to have the highest flavonoid content. Cell death induced by H₂O₂ was dose-dependently inhibited by the pretreatment with the ethyl acetate fraction. *O. stamineus* not only increased the expression of Bcl-2, but also decreased the expression of Bax, and ultimately reduced H₂O₂-induced apoptosis. Outcome of the search indicated that the antiapoptotic effect of...
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Anti-inflammatory and analgesic activities

The cell and animal model studies have provided strong evidence supporting the traditional use of _O. aristatus_ as a therapy for inflammatory disorders. Active constituents such as EUP, SIN, and ursolic acid were found to be the key anti-inflammatory agents. Yam _et al._[20] studied and investigated the anti-inflammatory activity on chemical constituents of the fractionated chloroform extract. Different techniques were applied to investigate the anti-inflammatory effect: anti-peritoneal capillary permeability, _in vitro_ nitric oxide (NO) scavenging activity, and carrageenan-induced hind paw edema in rats. The results showed that oral administration of the flavonoid-rich chloroform fraction at 500 and 1000 mg/kg reduced edema and caused NO and dye leakage to the peritoneal cavity. Phytochemical screening of the fraction confirms the presence of SIN and EUP. In another research, Akowuah and Zhari[47] used rat and mouse models to carry out both anti-inflammatory and analgesic activities of standardized 50% methanol extract of _O. stamineus_. The resulted showed that oral administration of up to 1000 mg/kg of the extract produced an anti-inflammatory effect as established by a reduction in the hind paw edema in rats pretreated with carrageenan. The analgesic activity was confirmed using the acetic acid–induced writhing test and formalin-induced licking test (late phase) in mice and rats; although oral administration of higher dose of the extract up to 1000 mg/kg did not show any effect on the tail flick and hot plate tests in mice. In another study, it was found that natural compounds isolated from _O. stamineus_ inhibit NO production in rats.[48] Although NO is an important signaling molecule, its excessive production triggers tissue damage and release of pro-inflammatory cytokines such as tumor necrosis factor, interferon, and interleukin.[48]

Antioxidant property

A number of studies showed that _O. stamineus_ has excellent antioxidant property. A research conducted by _et al._[30] showed that different kinds of extracts of _O. stamineus_ (distilled water, 50% aqueous methanol, methanol, 70% aqueous acetone, and chloroform extracts) produced different radical scavenging activities, using a 1,1-diphenyl-2-picrylhydrazyl _in vitro_ model system. The acetone extract showed the highest activity. Another study showed variations in the total phenolic compounds, ranging from 6.7 to 10.1 mg caffeic acid/g dry weight of the methanol extract. They also proved using different _in vitro_ methods (superoxide scavenging and xanthine oxidase) that _O. stamineus_ extract showed potential antioxidant activity.[17,49] _et al._[50] in another experiment investigated the antioxidative potency of various fractions of _O. stamineus_ extract using an _in vitro_ model of 1,1-diphenyl-2-picrylhydrazyl scavenging. The results showed antioxidant potency comparable to that of some standard antioxidants, including quercetin and butylated hydroxyanisole. The highest antioxidant activity showed by acetone extract was more than that of the aqueous methanol, methanol, and chloroform extracts. In another study, _et al._[51] demonstrated the antioxidant potency of the methanol extract of _O. stamineus_ using 1,1-diphenyl-2-picrylhydrazyl radical scavenging, Fe^{2+}-induced lipid peroxidation inhibiting activities, and trolox equivalent antioxidant capacity in _in vitro_ models. In a recent study, ethanol and aqueous extracts of _O. stamineus_ were evaluated _in vitro_ for their antioxidant, antimicrobial as well as their immunomodulatory properties on human peripheral blood mononuclear cells.[52] The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method, whereas the antibacterial effectiveness was carried out by both disc diffusion method and minimum inhibitory concentration (MIC) against four bacterial strains (gram-positive and gram-negative). In addition, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used for the investigation of immunomodulatory effect of the extracts. The result showed that aqueous extract of _O. stamineus_ exhibited significant free radical scavenging activity with IC_{50} of 9.6 µg/mL, whereas the IC_{50} for the ethanol extract was 21.4 µg/mL. These results showed that _O. stamineus_ showed high antioxidant activity and could be considered as an immunomodulatory agent also.[52] In another study, an ultrasound-assisted extraction (UAE) with ethanol was used to extract the compounds responsible for the antioxidant activities of _misai kucing_ _O. stamineus_).[53] For the optimization of four independent variables such as ethanol concentration (%), amplitude (%), duty cycle (W/s), and extraction time (min), response surface methodology (RSM) was used. Based on the optimal conditions, the experimental values were reported to be close to the predicted value by RSM modeling (P > 0.05), which showed the suitability of UAE for extracting the antioxidants of _misai kucing_. RA, kaempferol-rutinoside, and SIN were identified by HPLC–mass spectrometry.[53] The different antioxidant activities are mentioned in Table 1.

Antimicrobial activity

_O. stamineus_ is rich in phenolic secondary metabolites, and some of them also have antimicrobial activity. An _in vitro_ study using a disc diffusion assay showed that the extracts of this plant have antimicrobial activity against the selected foodborne bacteria.[58]
The variable antibacterial activity was shown when 50% methanol extract of *O. stamineus* was tested against *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Salmonella enteritidis*, *S. typhimurium*, and *Klebsiella pneumoniae*, with the highest growth inhibitory action seen against *V. parahaemolyticus*, a bacterium that causes mild gastroenteritis in humans on consumption of contaminated seafood. *O. stamineus* has excellent antimicrobial properties. The extract showed antibacterial activity on serotypes c and d of *Streptococcus mutans* (MIC = 7.8–23.4 mg/mL). However, later on, it was found that when treated in the presence of 5% sucrose, the potency gets decreased to approximately one-half, but no change was found in other type. The 50% methanol extract of *O. stamineus* inhibited *B. subtilis*, *B. cereus*, *S. aureus*, *L. monocytogenes*, *E. coli*, *V. parahaemolyticus*, *S. typhimurium*, and *K. pneumoniae*. [20]

The antimicrobial activities are given in Table 2.

### Hepatoprotective Activity

*O. stamineus* extract was found to reduce the necrotic changes in rat liver, and it inhibited the elevation of serum alanine transaminase and aspartate transaminase levels when treated with different doses (125, 250, 500, and 1000 mg/kg). The hepatoprotective effect was caused by its antioxidant and free radical scavenging properties. [51] In another study, methanol extract of leaves at a dose of 200 mg/kg showed hepatoprotective activity on paracetamol-induced rats. It is indicated that these properties were due to the ability to prevent the depletion of the tissue glutathione level. [60] As far as its aqueous extract is concerned, it lowered bilirubin level in jaundiced rat. This may be due to the increasing activity of glucuronyl transferase that facilitated hepatic conjugation of bilirubin or increased bilirubin binding by albumin. [61] Yam et al. [51] carried out an experiment on the methanolic extract of *O. stamineus* and reported its hepatoprotective effect. Alshawsh et al. [52] and Maheswari et al. [60] showed the hepatoprotective effect of ethanolic and methanolic extracts by using rat models for CCl₄, thioacetamide-, and paracetamol-induced hepatotoxicity, respectively. The results of liver function tests and histology studies monitored the progression of hepatotoxicity. These studies supported the hepatoprotective effect of this extract.

### Antihypertensive, hypoglycemic, hypolipidemic, and anti-obesity activities

Many works have been reported of antihypertensive activity of *O. stamineus*. A study showed that methylripaorichromene A (100 mg/kg) isolated from the leaves of *O. stamineus* decreased systolic blood pressure and heart rate, when it was injected subcutaneously into conscious male spontaneously hypertensive rats. [61] It also showed a concentration-dependent clamp down of retentions induced by high potassium, phenylephrine, or prostaglandin F₂α in endothelium-denuded rat thoracic aorta. Furthermore,
it exhibited a noticeable overpowering of contractile force without a noteworthy reduction in the heart rate in isolated bilateral guinea pig atria (negative inotropic effect). Lastly, it increased urine flow rate and absolute excretion of sodium, potassium, and chloride for 3 h after oral administration to saline-preloaded fasted rats. All these outcomes specify that methylripariochromene A of *O. stamineus* possesses an antihypertensive property considered to cause vasodilation, decreased cardiac output, and diuresis. Mariam et al.[62] evaluated the acute effects of aqueous *O. stamineus* extract on blood glucose levels in both normal and diabetic rats. The results showed that administration of *O. stamineus* aqueous extract (OSAE) at 1000 mg/kg produced hypoglycemic and antihyperglycemic effects in normal and streptozotocin (STZ)-induced diabetic rats, respectively. In this experiment, 14-day oral treatment was carried out with an aqueous extract of *O. stamineus* on plasma glucose and lipid profiles in normal and STZ-induced diabetic male Wistar rats. They reported reduction of plasma glucose levels in both euglycemic and hyperglycemic animals on oral administration of the extract at 200–1000 mg/kg. Overall results showed that OSAE is effective as an antihyperglycemic and an antihyperlipidemic agent in diabetic rats. Matsubara et al.[64] remarked the excellency of this plant in the treatment of hypertension. In another experiment, a combined nutraceutical study containing alcoholic extract of *O. stamineus* was carried out. Leaves reduced the systolic and diastolic blood pressure as well as pulse pressure of the patients with hypertensive dyslipidemia after 8 weeks of treatment. The nutraceutical tested contained berberine, monacolin, and policosanols, in addition to *O. stamineus* extract.[63] In another study, 50% methanolic extract was orally administered to spontaneous hypertensive rats at doses of 250, 500, and 1000 mg/kg BW. Systolic blood pressure of the rats decreased from 150 to 114 mmHg after 2 weeks’ treatment of dosing. Potency of the extract was comparable to irbesartan that was administered at 20 mg/kg BW in the positive control group. Antihypertensive effects of the extract were planned to be facilitated by diuresis and natriuresis.[64] Recently Azam et al.[65] reported that aqueous, ethanolic, 50% aqueous ethanolic and methanolic extract, when given orally at a dose of 500 mg/kg body weight (bw) for 14 days to diabetic rats induced via intraperitoneal injection of 60 mg/kg bw STZ showed OS aqueous extract (OSAE) caused a reversal of diabetes mellitus comparable to that of 10 mg/kg bw glibenclamide. Nuclear magnetic resonance metabolomics approach using pattern recognition combined with multivariate statistical analysis was applied in the rat urine to study the effect. A total of 15 urinary metabolites, whose levels changed significantly on treatment, were identified as the biomarkers of OSAE in diabetes. Another recent study conducted by Seyedan et al.[66] reported antiglycemic effect of *O. stamineus*. In this experiment, ethanolic extract of leaves of *O. stamineus* (200 and 400 mg/kg) and its major compound (RA, 10 mg/kg) in obese mice (C57BL/6) induced by a high-fat diet with continuous supplementation with *O. stamineus* extract (200 and 400 mg/kg) for 8 weeks considerably reduced BW gain. However, supplementation with RA, a constituent in the extract, produced only a slight reduction in BW gain compared to the high-fat diet control group. Result showed that the ethanolic extract of leaves of *O. stamineus* can significantly reduce a gain in BW and possess hypolipidemic and anti-obesity effects, thus guarding against the adverse effects of high-fat diet-induced obesity. Recently, Shafaei et al.[67] reported *in vitro* angiotensin-converting enzyme (ACE) inhibition activity of different extracts of *O. stamineus* leaves and their main flavonoids, namely RA, SIN, EUP, and 3′-hydroxy-5,6,7,4′-TMF. The *in vitro* ACE inhibition activity relied on determining the hippuric acid formation from ACE-specific substrate (hippuryl–histidyl–leucine) by the action of ACE enzyme.

**Gastroprotective activity**

The Malay traditional uses of *O. stamineus* in the treatment of gastric ailments are compelling reasons for investigating its possible gastroprotective effect.[68] In an experiment, the anti-ulcerogenic activity of 50% methanol extract of *O. stamineus* leaves was evaluated against ethanol-induced ulcers in male Sprague Dawley rats. The results exposed a significant dose-dependent (125, 250, 500, and 1000 mg/kg) decrease in the ulcer index (UI) and gastric mucosal damage and lipid peroxidation along with an increase in mucus secretion. It was established that the extract possesses a gastroprotective property that is endorsed by its ability to inhibit lipid peroxidation and stimulate gastric mucus secretion to the high concentration of RA. In another *in vivo* model experiment, the anti-ulcer activity of *Orthosiphon* leaves extract was evaluated through several parameters that involve gastric acidity, number of ulcers, diameters of ulcers, UI, and healing ratio. The dose levels of *Orthosiphon* leaves extract that was used in this study were 250 and 500 mg/kg, respectively. The results showed that *Orthosiphon* leaves extract have significantly different gastric ulcer healing properties when compared to control group. It was also supported by histopathological exmination.[69]
CONCLUSION

*O. stamineus* Benth. is a valued medicinal plant, growing well in many countries, especially Southeast Asian countries. This plant has a great potential value for cultivation because it contains secondary metabolites with interesting biological activities. Numerous compounds have been isolated from this plant, and this species has been used in the treatment and prevention of several illnesses such as diarrhea, inflammation, and intestinal disorders. Many experiments have been conducted to validate its pharmacological uses. This review has presented a comprehensive overview on the phytochemistry, and phytochemical and pharmacological applications of *O. stamineus* and its main compounds. In addition, clinical evaluation of the possible toxicity related to these isolated compounds needs to be assessed for finding their application as a biotherapeutical medicine.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Indubala J, Ng LT. Herbs: the green pharmacy of Malaysia. Kuala Lumpur, Malaysia: Vipress; 2000. p. 76-7.
2. Hegnauer R. Chemotaxonomic der Pflanzen, Vol. IV, Birkhäuser Verlag, Stuggart. 1966. pp. 314-6.
3. Wangner H. Pharmacetic biology: drugs and their inhalants. 2nd ed. Stuttgart, Germany: Gustav Fischer; 1982. p. 45-90.
4. Eisai PT. Indonesia Medicinal Herb Index in Indonesia, 2nd ed.; University Press: Godjah, Mada, 1995. pp. 239-63.
5. Chung WG, Roh HK, Kim HM, Cha YN. Monooxygenase in N-demethylation of caffeine; identified by using inducer treated rat liver microsomes that are characterized with testosterone metabolic patterns. Chem Biol Interact 1998;113:1-14.
6. Masuda T, Masuda K, Shiragami S, Jitoe A, Nakatani N. Orthosiphon A and B, novel diterpenoid inhibitors of TPA (12-O-tetradecanoylphorbol–13-acetate)–induced inflammation, from *Orthosiphon stamineus*. Tetrahedron 1992;48:6787-92.
7. Beaux D, Fleurentin J, Mortier F. Effect of extracts of *Orthosiphon stamineus* Benth., *Hieracium pilosella* L., *Sambucus nigra* L., and *Arctostaphylos uva-ursi* (L.) Spreng. in rats. Phytother Res 1999;13:222-5.
8. Tezuka Y, Stampoulis P, Banskota AH, Awale S, Tran KQ, Saiki I, et al. Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*. Chem Pharm Bull 2000;48:1711-9.
9. Sumaryono W, Proksch P, Wray V, Witte L, Hartmann T. Qualitative and quantitative analysis of the phenolic constituents from *Orthosiphon aristatus*. Planta Med 1991;57:176-80.
10. Olah NK, Radu L, Mogoșan C, Hanganu D, Gocan S. Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth. (Lamiaceae) hydroalcoholic extracts. J Pharm Biomed Anal 2003;33:117-23.
11. Taleeda Y, Matsumoto T, Terao H, Shingu T, Futatsushi Y, Nohara T, et al. Orthosiphol D and E, minor diterpenes from *Orthosiphon stamineus*. Phytochemistry 1993;33:411-5.
12. Yam MF, Asmawi MZ, Basir R. An investigation of the anti-inflammatory and analgesic effects of *Orthosiphon stamineus* leaf extract. J Med Food 2008;11:362-8.
13. Yam MF, Ang LF, Basir R, Salman IM, Ameer OZ, Asmawi MZ. Evaluation of the anti-pyretic potential of *Orthosiphon stamineus* Benth. standardized extract. Inflammopharmacology 2009;17:50-4.
14. Yuliana ND, Khatib A, Link-Struensee AMR, Ijzerman AP, Rungkat-Zakaria F, Young HC, et al. Adenosine A1 receptor binding activity of methoxy flavonoids from *Orthosiphon stamineus*. Planta Medica 2009;75:132-6.
15. Mohamed EA, 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;16:787-801.
16. 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;547:439-42.
17. Akowuah GA, Zhari I, Norhayati I, Sadikun A, Khamsham 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;87:559-66.
18. 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;93:311-7.
19. Hossain MA, Mizanur Rahman SM. Isolation and characterisation of flavonoids from the leaves of medicinal plant *Orthosiphon stamineus*. Arab J Chem 2015;8:218-21.
20. Yam MF, Lim V, Salman IM, Ameer OZ, Ang LF, Rosidah N, et al. HPLC and anti-inflammatory studies of the flavonoid rich chloroform extract fraction of *Orthosiphon stamineus* leaves. Molecules 2010;15:4452-66.
21. Banskota AH, Tezuka Y, Le Iran Q, Kadota S. Chemical constituents and biological activities of Vietnamese medicinal plants. Curr Topics Med Chem 2003;3:227-48.
22. 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;27:328-34.
23. Awale S, Tezuka Y, Banskota AH, Ketut Adnyana I, Kadota S. Highly-oxygenated isopimarane-type diterpenes from *Orthosiphon stamineus* of Indonesia and their nitric oxide inhibitory activity. Chem Pharma Bull 2003;51:268-75.
24. Awale S, Tezuka Y, Banskota AH, Kouda K, Tun KM, Kadota S. Four highly oxygenated isopimarane-type diterpenes of *Orthosiphon stamineus*. Planta Medica 2002;68:286-8.
25. Shibuya H, Bolgaki T, Ohashi K. Two novel migrated pimarane-type diterpenes, neoorthosiphols A and B, from the leaves of *Orthosiphon aristatus* (Lamiaceae). Chem Pharma Bull 1991;47:911-2.
26. Awale S, Tezuka Y, Kobayashi M, Ueda Y, Kadota S. Neoorthosiphonone A; a nitric oxide (NO) inhibitory diterpene with new carbon skeleton from *Orthosiphon stamineus*. Tetrahedron Lett 2004;45:1359-62.
27. Stampoulis P, Tezuka Y, Banskota AH, Tran KQ, Saiki I, Kadota S. Staminolactones A and B and nor staminol A: three highly oxygenated staminane-type diterpenes from *Orthosiphon stamineus*. Organic Lett 1999;1:1367-70.

28. Misuda T, Masuda K, Nakatani N. Orthosiphol A: a highly oxygenated diterpene from the leaves of *Orthosiphon stamineus*. Tetrahedron Lett 1992;33:945-6.

29. Nguyen MT, Awale S, Tezuka Y, CMen-Hsiung C, Kadota S. Siphonols A-E: novel nitric oxide inhibitors from *Orthosiphon stamineus* and their structure-activity relationship. Biol Pharma Bull 2003;26:468-73.

30. 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 Pharma Bull 2003;26:468-73.

31. Awale S, Tezuka Y, Banskota AH, Kadota S. Nitric oxide inhibitors from *Orthosiphon stamineus* of Indonesia. Bioorganic Med Chem Lett 2003;13:31-5.

32. Stampoulis P, Tezuka Y, Banskota AH, Kim Qui T, Saiki I, Kadola S, et al. A novel diterpene from *Orthosiphon stamineus*. Tetrahedron Lett 1999;40:4239-42.

33. Hossain MA, Ismail Z. Isolation and characterization of triterpenes from the leaves of *Orthosiphon stamineus*. Arab J Chem 2013;6:295-8.

34. Sair NS, Abdalrahim AFA, Hamil MSR, Abdul Majid AMS, Ismail Z. A novel reverse phase high-performance liquid chromatography method for standardization of *Orthosiphon stamineus* leaf extracts. Pharmacognosy Res 2015;7:23-31.

35. Hashim S, Beh HK, Hamil MS, Ismail Z, Majid AM. High-performance thin-layer chromatography method development, validation, and simultaneous quantification of four compounds identified in standardized extracts of *Orthosiphon stamineus*. Pharmcognosy Res 2016;8:238-43.

36. See Tiam Y, Tee Siau N, Ang Teck CH, Chan R, Yusoff C, Ngoh Gc C. Assessment of various pretreatment and extraction methods for the extraction of bioactive compounds from *Orthosiphon stamineus* leaf via microstructures analysis. Int J Food Eng 2016;12:711-7.

37. Sree NV, Sri PU, Ramaroa N. Neuro-protective properties of *Orthosiphon stamineus* (Benth) leaf methanolic fraction through antioxidant mechanisms on SH-SY5Y cells: an in-vitro evaluation. Int J Pharm Sci Res 2015;6:1115-25.

38. Dolečková I, Rárová L, Grúz J, Vondrusová M, Strnad M, Kryštof V. Antiproliferative and antiangiogenic effects of flavone eupatorin, an active constituent of chloroform extract of *Orthosiphon stamineus* leaves. Fitot 2012;83:1000-7.

39. Awale S, Tezuka Y, Banskota AH, Kadota S. Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. J Nat Prod 2001;64:392-6.

40. Ahmed MBK, Aisha AFA, Nassar ZD, Siddiqui JM, Ismail Z, Omari SMS, et al. Cat’s whiskers tea (*Orthosiphon stamineus*) extract inhibits growth of colon tumor in nude mice and angiogenesis in endothelial cells via suppressing VEGFR phosphorylation. Nutri Cancer 2012;64:89-99.

41. Salleh SA, Rajab NE, Abdullah NR, Ismail Z, Mouatt P, Dowell A, et al. *In vitro* chemopreventive activity of an ethyl acetate fraction derived from hot water extract of *Orthosiphon stamineus* in HepG2 cells. J Med Plants Res 2011;5:1892-9.

42. Sundaramal S, Thirugnanasampandan R, Selvi MT. Chemical composition analysis and antioxidant activity evaluation of essential oil from *Orthosiphon thyminiforus* (Roth.) Slessen. Asian Pac J Trop Biomed 2012;5:112-5.

43. Awale S, Tezuka Y, Banskota AH, Shimoji S, Taira K, Kadota S. Norstaminane- and isopimarane-type diterpenes of *Orthosiphon stamineus* from Okinawa. Tetrahedron 2002;58:5503-12.

44. Sahib HB, Ismail Z, Othman NH, Abdul Majid AMS, *Orthosiphon stamineus* Benth. methanolic extract enhances the anti-proliferative effects of tamoxifen on human hormone dependent breast cancer. Inter J Pharmacol 2009;5:273-6.

45. Abdelwahab SI, Mohan S, Elhassan MM, Al-Mekhlafi N, Mariod AA, Abdul AB, et al. Antiapoptotic and antioxidant properties of *Orthosiphon stamineus* Benth (cat’s whiskers): intervention in the Bel-2-mediated apoptotic pathway. Evid Based Complement Alternat Med 2011;2011:156765.

46. Hsu CL, Hong BOH, Shan YU, Yen GC. Antioxidant and anti-inflammatory effects of *Orthosiphon aristatus* and its bioactive compounds. J Agric Food Chem 2010;58:2150-6.

47. Akowuah GA, Zhari I. Effect of extraction temperature on stability of major polyphenols and antioxidant activity of *Orthosiphon stamineus* leaf. J Herbs Spices Med Plant 2010;16:160.

48. Kuo PC, Schroeder RA. The emerging multifaceted roles of nitric oxide. Ann Surg 1995;221:220-35.

49. Akowuah GA, Zhari I, Sadikun A, Norhayati I. HPTLC densitometric analysis of *Orthosiphon stamineus* leaf extracts and inhibitory effect on xanthine oxidase activity. Pharm Biol 2006;44:65-70.

50. Akowuah GA, Zhari I, Norhayati I, Sadikun A. Radical scavenging activity of methanol leaf extracts of *Orthosiphon stamineus*. Pharm Biol 2005;42:629-35.

51. Yam MF, Basir R, Asmawi MZ, Ismail Z. Antioxidant and hepatoprotective properties of *Orthosiphon stamineus* Benth. standardized extract. Am J Chin Med 2007;35:115-26.

52. Alshawsh MA, Abdulla MA, Ismail S, Amin ZA, Qader SW, Hadi HA, et al. Free radical scavenging, antimicrobial and immunomodulatory activities of *Orthosiphon stamineus*. Molecules 2012;17:5385-95.

53. Lin YT, Labge RG, Shetty K. Inhibition of *Vibrio parahaemolyticus* in seafood systems using oregano and cranberry phytochemical synergies and lactic acid. Innovat Food Sci Emerging Technol 2005;6:453-8.

54. Chew KK, Khoo MZ, Ng SY, Thoo YY, Aida WMW, Ho CW. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. Int Food Res J 2011;18:1427-35.

55. Farhan M, Abdul Raza S, Pin KY, Chua AL. Antioxidant activity and phenolic content of different parts of *Orthosiphon stamineus* grown under different light intensities. J Tropical Forest Sci 2012;24:173-7.

56. Ho CH, Noryati I, Sulaiman SF, Rosma A. Antimicrobial properties of *Orthosiphon stamineus* extract against food-borne bacteria. Food Chem 2001;74:375-82.

57. Oleníková DN, Tankhaeva LM. Physicochemical characteristics and antioxidant activity of melatonin pigment from the fermented leaves of *Orthosiphon stamineus*. Brazilian J Pharmacog 2011;22:284-90.

58. Ho SK, Tan CP, Thoo YY, Abas F, Ho CW. Ultrasound-assisted extraction of antioxidants in misai kucing (*Orthosiphon aristatus*). Molecules 2014;19:21640-59.

59. Laikangbam R, Devi MD, Singh SR. Anti-bacterial activity and inhibitory effect on xanthine oxidase activity of *Orthosiphon stamineus* leaf extracts against food-borne bacteria. Food Chem 2010;122:1168-72.

60. Maheswari K, Marymml R, Venkatanarayana R. Hepatoprotective activity of *Orthosiphon stamineus* on liver...
damages caused by paracetamol in rats. Jordan J Biol Sci 2008;1:105-8.
61. Matsubara T, Bohgaki T, Watarai M, Suzuki H, Ohashi K, Shibuya H. Antihypertensive actions of methylripariochromene A from Orthosiphon aristatus, an Indonesian traditional medicinal plant. Biol Pharm Bull 1999;22:1083-8.
62. Mariam A, Amawi MZ, Sadikun A. Hypoglycaemic activity of the aqueous extract of Orthosiphon stamineus. Fitoterapia 1996;67:465-8.
63. Cicero AFG, De Sando V, Izzo R, Vasta A, Trimarco A, Borghi C. Effect of a combined nutraceutical containing Orthosiphon stamineus effect on blood pressure and metabolic syndrome components in hypertensive dyslipidemic patients: a randomized clinical trial. Complement Ther Clin Pract 2012;18:190-1.
64. Azizan NA, Ahmad Mohamed K, Ahmad MZ, Asmawi Z. The in vivo antihypertensive effects of standardized methanol extracts of Orthosiphon stamineus on spontaneous hypertensive rats: a preliminary study. African J Pharmacol 2012;6:376-9.
65. Azam AA, Pariyani R, Safinar I, Ismail A, Khatib A, Abas F, et al. Urinary metabolomics study on the protective role of Orthosiphon stamineus in streptozotocin induced diabetes mellitus in rats via 1H NMR spectroscopy. BMC Complement Altern Med 2017;17:278.
66. Seyedan A, Alshawsh MA, Alshagga MA, Mohamed Z. Antiobesity and lipid lowering effects of Orthosiphon stamineus in high-fat diet-induced obese mice. Planta Med 2017;83:684-92.
67. Shafaei A, Khan S, Aisha AFA, Abdul Majid AMS, Hamdan MR, Mordi MN, et al. Flavonoids-rich Orthosiphon stamineus extract as new candidate for angiotensin I-converting enzyme inhibition: a molecular docking study. Molecules 2016;21:1500.
68. Yam MF, Ang LF, Salman IM, Ameer OZ, Lim V, Ong LM, et al. Orthosiphon stamineus leaf extract protects against ethanol-induced gastropathy in rats. J Med Food 2009;12:1089-97.
69. Yuniarto A, Susilawati E, Ismi Khairunnisa I, Dadang Juanda D, Finna Setiawan F. Antioxidant and gastric ulcer healing effect of Orthosiphon stamineus (Benth.) leaves extract in aspirin-induced rats. Asian J Pharm Clin Res 2017;10:397-9.