EFFECT OF ORGANIC SOIL AMENDMENTS ON GROWTH, PHYTOCHEMICAL CONTENT AND ANTIMICROBIAL ACTIVITY OF MISAI KUCING (*Orthosiphon stamineus*)

CHEE HOYAU

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ABSTRACT

Extensive research has been carried out on Misai Kucing (*Orthosiphon stamineus* Benth) from the family of Lamiaceae since 1930s. It has been widely used as herbal tea called 'Java tea' in Asian and European countries with various types of pharmacological properties and medicinal values. An experiment was conducted at Faculty of Sustainable Agriculture in Universiti Malaysia Sabah to investigate the effect of organic soil amendments on growth and yield, phytochemical content, antioxidant and antimicrobial activity of *Orthosiphon stamineus.* This is due to the lack of studies regarding the effect of plant-based organic soil amendments on secondary metabolites in *O. stamineus* plant. The experiment was conducted under the rain shelter of the campus in polybags filled with topsoil, sand and organic matter at a ratio of 3:2:1, in which the organic matters were the treatments used. Plant samples were harvested after 12 weeks of planting with plant growth parameters measured weekly and the samples were extracted for secondary metabolites analysis. The experimental design used in this study was completely randomised design (CRD), using spent mushroom substrate (SMS), rice husk, empty fruit bunch (EFB), rice bran and cocopeat as organic soil amendment treatments with five replicates each. Data were analysed with one way ANOVA using SPSS software and the treatment means were compared by Tukey's test at 5% of significance level. Generally, *Orthosiphon stamineus* plants treated with organic soil amendments showed significant difference in growth and yield over control medium. The best performance in growth and yield of *O. stamineus* resulted from EFB amendment planting medium. Total phenolic content of *O. stamineus* was the highest for planting medium treated with rice husk with value of 16.35 ± 2.86 mg GAE/g while total flavonoid content of plant was the highest in planting medium treated with spent mushroom substrate (SMS) with value of 13.43 ± 1.12 mg QE/g. Antioxidant activity of *O. stamineus* was best performed in planting medium treated by rice husk, which showed IC_{50} inhibition value of 5301.14 ± 389.38 µg/ml. For antimicrobial activity analysis, *O. stamineus* was found to have inhibition against bacteria *Escherichia coli* and *Bacillus cereus* only without significant difference among treatments. The amendments of rice husk and SMS in planting medium has a potential to improve phytochemical content and antioxidant activity in *O. stamineus*.

KESAN PENAMBAH TANAH ORGANIK KE ATAS PERTUMBUHAN, KANDUNGAN FITOKIMIA DAN AKTIVITI ANTIMIKROB MISAI KUCING (Orthosiphon stamineus)

ABSTRAK

Kajian menyeluruh telah dijalankan ke atas Misai Kucing (Orthosiphon stamineus Benth) dari keluarga Lamiaceae sejak 1930-an. Ia telah digunakan secara meluas sebagai teh herba yang dikenali sebagai 'Teh Java' di negara-negara Asia dan Eropah dengan pelbagai sifat famakologi dan nilai-nilai perubatannya. Eksperimen ini telah dijalankan di Fakulti Pertanian Lestari di Universiti Malaysia Sabah untuk mengkaji kesan penambah tanah organik ke atas pertumbuhan dan hasil, kandungan fitokimia, aktiviti antioksidan dan antimikrob Orthosiphon stamineus. Hal ini disebabkan kajian tentang kesan penggunaan bahan tumbuhan sebagai asas penambah tanah organic terhadap metabolit sekunder tumbuhan O. stamineus masih kekurangan. Kajian ini telah dijalankan di bawah pelindung hujan di kampus di dalam polibag yang diisi dengan tanah, pasir dan bahan organik dengan nisbah 3:2:1, di mana bahan organik tersebut merupakan rawatan yang digunakan. Bahan tanaman telah dituai 12 minggu selepas penanaman dan parameter pertumbuhan tanaman telah diukur secara mingguan. Sampel tanaman diekstrak kemudian bagi tujuan analisis metabolit sekunder. Reka bentuk eksperimen yang telah digunakan adalah reka bentuk rawak lengkap dengan menggunakan sisa substrat cendawan, sekam padi, serat tandan kosong kelapa sawit, dedak padi dan gambut kelapa sebagai rawatan penambah tanah organik. Lima replikasi telah digunakan bagi setiap jenis rawatan. Data telah dianalisa dengan perisikan SPSS menggunakan ANOVA satu hala dan keputusan kajian telah diuji dengan kajian Tukey pada aras signifikan 5%. Secara keseluruhannya, tumbuhan O. stamineus yang dirawat dengan penambah tanah organik telah menunjukkan perbezaan yang signifikan terhadap pertumbuhan and hasil tumbuhan jika dibandingkan dengan tumbuhan tanpa rawatan. Pertumbuhan dan hasil tumbuhan yang terbaik adalah tumbuhan yang dirawat dengan serat tandan kosong kelapa sawit. Jumlah kandungan fenolik O. stamineus didapati tertinggi pada tumbuhan yang dirawat dengan media penambah tanah sekam padi dengan nilai 16.35 ± 2.86 mg GAE/g manakala jumlah kandungan flavonoid tumbuhan didapati tertinggi pada tumbuhan yang dirawat dengan media penambah tanah sisa substrat cendawan dengan nilai 13.43 ± 1.12 mg QE/g. Aktiviti antioksidan O. stamineus yang terbaik adalah didapati pada tumbuhan yang dirawat dengan sekam padi yang menunjukkan nilai kepekatan perencatan 50 5301.14 ± 389.38 µg/ml. Bagi aktiviti antimikrob, adalah didapati bahawa O. stamineus mempunyai perencatan terhadap bacteria Escherichia coli and Bacillus aureus sahaja tanpa perbezaan yang signifikan antara semua rawatan. Penambah tanah sekam padi dan sisa substrat cendawan berpotensi untuk meningkatkan kandungan fitokimia dan aktiviti antioksidan O. stamineus.

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A_C	Absorbance of control
ANOVA	Analysis of variance
A_S	Absorbance of tested sample
CBSC	Carbon-based secondary compounds
CRD	Completely Randomised Design
CRH	Carbonised rice husk
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Electrical conductivity
EFB	Empty fruit bunch
FRSA	Free radical scavenging activity
FSA	Faculty of Sustainable Agriculture
FYP	Final Year Project
GAE	Gallic acid equivalents
H_A	Alternative hypothesis
H_o	Null hypothesis
HPLC	High-performance liquid chromatography
IC_{50}	Inhibitory concentration 50
LSD	Least Significant Difference
MDF	Medium density fibrebooard
OH	Hydroxyl group
PAL	Phenyl alanine lyase
RE	Rutin equivalent
RHA	Rice husk ash
ROS	Reactive oxygen species
Rpm	Rotation per minute
SOM	Soil organic matter
SPSS	Statistical Package for the Social Sciences
TFC	Total flavonoid content
TPC	Total phenolic content
UMS	Universiti Malaysia Sabah
UTI	Urinary tract infection
VEGF	Vascular endothelial growth factor
WSA	Water-stable aggregates

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

Misai Kucing plant (*Orthosiphon stamineus* Benth) which is also called Cat's Whiskers belongs to family Lamiaceae. The word *Orthosiphon* is derived from two Latin words, "orthos" and "siphon" which means straight while cylindrical. Misai Kucing can grow to a height of 1.5 m and can be harvested in three to four months after propagating its stem cutting. This plant is a medicinal herbaceous shrub which is widely distributed in Africa and Southeast Asia such as Thailand, Indonesia, the Phillipines and Brunei. The plant can be identified by its purple- or white-coloured flowers that resemble cat's whiskers. In Asia, the leaves of this herbal plant are widely used in the form of herbal tea which is popularly known as 'Java tea'. Java tea was possibly introduced to the West in the early 20^{th} century (Jaganath *et al.*, 2000).

The medicinal properties of *Orthosiphon stamineus* have been proven in the literature by numbers of active compound isolated and identified from the extract. Traditionally, the leaves of this plant can treat several primary health ailments such as rheumatism, abdominal pain, kidney and bladder inflammation, edema and gout. Besides, studies have shown that the leaves of this herbal plant exhibit a range of pharmacological properties such as anti-inflammatory, antioxidant, anti-bacterial, anti-angiogenetic properties (Beaux *et al.*, 1999; Tezuka *et al.*, 2000).

Orthosiphon stamineus contains high level of phytochemicals, including the phenolic group such as total flavonoids (Amzad and Mizanur 2013), rosmarinic acid (Olah *et al.*, 2004; Akowuah *et al.*, 2004) and antioxidants (Ho *et al.*, 2010). The numerous flavonoid compounds along with some of the other compounds available in *O. stamineus* have qualified it to be one of the most effective antioxidant foodstuffs. A rapid, quantitative and simultaneous HPLC-based determination of major phytochemicals from the extract of *O. staminues* leaves has been reported and its strong antioxidant potency and total phenolic content have been reconfirmed (Akowuah *et al.*, 2005). The strong antioxidant action of the plant is what makes many researchers to explore its potential pharmacological properties.

In order to enhance growth and obtain optimal yield, nutrients must be provided to plants in an appropriate time, place, quantity and proportion in a usable form for plants. This requires the application of fertilizers or soil amendments. The primary role of soil amendments is to provide nutrients for growth of crops and also to provide materials for soil improvement. Based on reference guide of British-Columbia Environmental Farm Plan (2010), soil amendments are defined as all materials applied to the soil on farms as fertilizers or as soil conditioner. Sources of soil amendments can come from on-farm sources and off-farm sources. On farm amendments include bedding, compost, crop residue, spoiled feed, spent mushroom media, spent nutrient solution and so on. Off-farm amendments include chemical fertilizers, chemical conditioners such as lime, non-agricultural wastes such as municipal biosolids and others. A research from Marques *et al.* (2014) had proven that spent mushroom compost can act as a substrate for the growth and development of lettuce seedlings. Badar and Qureshi (2014) had also stated that composted rice husk can help improving the growth and biochemical parameters of sunflower plants by improving the organic content in soil.

Soil amendments have been reported to have an influence on the phyto-nutritional quality of crops. Inorganic fertilizer was found to reduce the antioxidant levels, while organic fertilizer had been proven to enhance antioxidant content in tomato plants (Dumas *et al.*, 2003). In previous study, a strawberry cultivar grown under organic conditions showed higher levels of phenolics than its inorganically grown counterparts (Hakkinen and Torronen, 2000). Asami *et al.* (2003) reported that there was a significantly higher total phenolics in marionberries applied with organic fertilizer when compared to inorganic fertilizer. Besides, Weibel *et al.* (2000) also stated that the phenol (mainly flavonols) content of apple cultivars grown organically was 19% higher than the apples grown inorganically. Researches showed that the application of organic fertilizers can boost up the production of plant secondary metabolites which include the phytochemicals stated as well.

1.2 Justification

This study was carried out to test and determine the effect of organic soil amendments on growth and yield, phytochemical content, antioxidant activity and antimicrobial activity on Misai Kucing plant (*Orthosiphon stamineus*).

Besides light intensity and temperature (Chua *et al.*, 2015), salinity of soil (Aghaei and Komatsu, 2013), harvesting period (Brasileiro *et al.* 2015), leaf positions (Bhakta and Ganjewala, 2009) and storage condition (Ismawaty *et al.*, 2015), soil amendment (Dumas *et al.*, 2003) is one of the factors that will affect the phytochemical content and antioxidant activity in plant. In fact, soil amendments can improve soil condition in various aspects, which is good for plant growth. As soil is the base and an essential element to grow plants, it is very important to apply soil of good quality and optimum for plant growth.

The impact of fertilizers on vegetative growth of this plant has been well documented. However, studies regarding the effect of different types of organic soil amendments on phytochemical content and antioxidant and antimicrobial activity in *O. stamineus* are still lacking. There have been a number of studies using animal manures such as chicken manure, cow manure or goat manure as treatments of organic fertilizers to improve plant growth. Yet, the use of plant-based materials as organic soil amendments is not common and not completely utilised. Instead of being treated as a waste, plant-based materials can be useful by adding to soil medium as amendments, which release certain amounts of nutrients besides improving soil conditions. This is to determine the ability and adaptability of plant residues in their contribution on improving soil fertility, thus boosting growth and indirectly secondary metabolites of the plant. On top of that, agricultural sustainability can be promoted. In this case, the utilisation of plant-derived materials as soil amendments can minimise or reduce the use of artificial or inorganic fertilizers on the plants, recycle unwanted farm residues and also reuse them to benefit the soil and plant growth. This reduces negative impacts that will be brought to the environment in long term.

1.3 Objectives

The objectives of this study are to determine

i. the effect of organic soil amendments on growth and yield of Misai Kucing (*Orthosiphon stamineus*).
ii. the effect of organic soil amendments on phytochemical content and antioxidant activity of Misai Kucing (*Orthosiphon stamineus*).
iii. the effect of organic soil amendments on antimicrobial activity of Misai Kucing (*Orthosiphon stamineus*).

1.4 Hypotheses

Hypothesis for objective (i):

H_o: There is no significant difference between different types of organic soil amendments on growth and yield of Misai Kucing (*Orthosiphon stamineus*).

H_a: There is significant difference between different types of organic soil amendments on growth and yield of Misai Kucing (*Orthosiphon stamineus*).

Hypothesis for objective (ii):

H_o: There is no significant difference between different types of organic soil amendments on phytochemical content and antioxidant activity of Misai Kucing (*Orthosiphon stamineus*).

H_a: There is significant difference between different types of organic soil amendments on phytochemical content and antioxidant activity of Misai Kucing (*Orthosiphon stamineus*).

Hypothesis for objective (iii):

H_o: There is no significant difference between different types of organic soil amendments on antimicrobial activity of Misai Kucing (*Orthosiphon stamineus*).

H_a: There is significant difference between different types of organic soil amendments on antimicrobial activity of Misai Kucing (*Orthosiphon stamineus*).

CHAPTER 2

LITERATURE REVIEW

2.1 Misai Kucing (*Orthosiphon stamineus* Benth)

The genus name Orthosiphon was coined from two Latin words, Orthos and siphon. The word Orthos referred to straight while siphon meant tube-like or cylindrical. These two words are actually referred to the straight tube-like flowers that were produced by the *Orthosiphon* spp. and this was considered as one of the main characteristics of the Labiatae or Lamiaceae family (Chan and Loo, 2006).

Another biological name of *Orthosiphon stamineus* Benth is *Ocimum aristatum.* Vernacular names of *Orthosiphon stamineus* is Misai Kucing or Kumis Kuching (Malay) (Prapti and Tim, 2003), Mao Xu Cao or Mao Xu Hua (Chinese), Cat's Whiskers, Java tea (English) and Elisenkat (Panope) (Kenneth and Damian, 1979).

Orthosiphon stamineus is a medicinal plant native to Southeast Asia (Malaysia, Indonesia, Thailand etc.) and some parts of tropical Australia. In Malaysia, *Orthosiphon stamineus* (known as Misai Kucing) is of interest as it is easily accessible and commonly consumed to treat various ailments (Khamsah *et al.*, 2006). Leaves of this plant are used commonly in Southeast Asia and European countries for herbal tea, well known as "Java tea" (Indubala and Ng, 2000). It is very popular in Southeast Asian folk medicine, where it is used extensively to treat diabetes, hypertension, rheumatoid diseases, epilepsy, menstrual disorder, gonorrhea, syphilis, renal calculus, gallstone, edema, eruptive fever, influenza, hepatitis, jaundice and others (Awale *et al.*, 2003). *O. stamineus* is very popular for its diuretic effect, which is stronger than most other natural diuretics (Burkill, 1966). Besides, it has been used as antihypertensive, anti-inflammatory, anti-allergic, and anti-cancer drug (Matsubara *et al.*, 1999).

2.2 Botanical Description of *Orthosiphon stamineus*

Orthosiphon stamineus is a herbaceous shrub which grows to a height of 1.5 meter and can be harvested in 3-4 months after propagating its stem cuttings. The plant is wild, which can be seen growing along the forest edges, roadsides and wasteland. The leaves are arranged in opposite pairs, lanceolate-like, rhomboid, simple, green, 2 to 4 cm wide and 4 to 7 cm long as shown in Figure 2.1 (A). The leaf apex is acuminate with acute leaf base and the petioles are short and reddish purple. The stem is quadrangular, erect, branching profusely and reddish-coloured as shown in Figure 2.1 (B). The inflorescence is campanulate in shape, white or purple in colour with long exerted filaments that look like cat's whiskers. Besides, it has stamens that extend from the corolla-tube with a length of more than 2 cm (Wiart, 2000). It has been cultivated for a long time and is popular for its elegant and unique flower, which is white and bluish in colour with far-exerted filaments that looks like cat's whiskers.

According to both the floral and calyx colours, *Orthosiphon sp.* is classified into one of two varieties (Lee, 2004): One with white flowers (white variety) as shown in Figure 2.1 (C) and the other with light purple flowers (purple variety). The purple variety has more bioactive compounds than the white one (Lee, 2004). However, most scientific investigations have used the white variety.

The purple variety and the white variety of cat's whiskers (*Orthosiphon stamineus* Benth) could be mistakenly considered as the same type if they are not carefully observed because most of their external morphological characters were similar. Studies had shown that these two varieties could possibly be identified by their floral and leaf morphology. The white variety produced rhomboid shaped leaves without any coloured spots with acuminate apex, obtuse base and light green venation. Contrarily, the purple variety produced ovate shape leaves with acute apex, truncate base and purple venation. Nevertheless, the two most distinct floral characters were the colours of corolla and calyx. The purple variety produced light purple tint at the lobes of the two-lipped corolla with maroon calyx while the petals of the white variety was totally white without purple tint at the edge of the petal lobes and green calyx. However, the micro-structures of leaves, stigma, anthers and pollen grains were the same for both varieties (Chan and Loo, 2006).

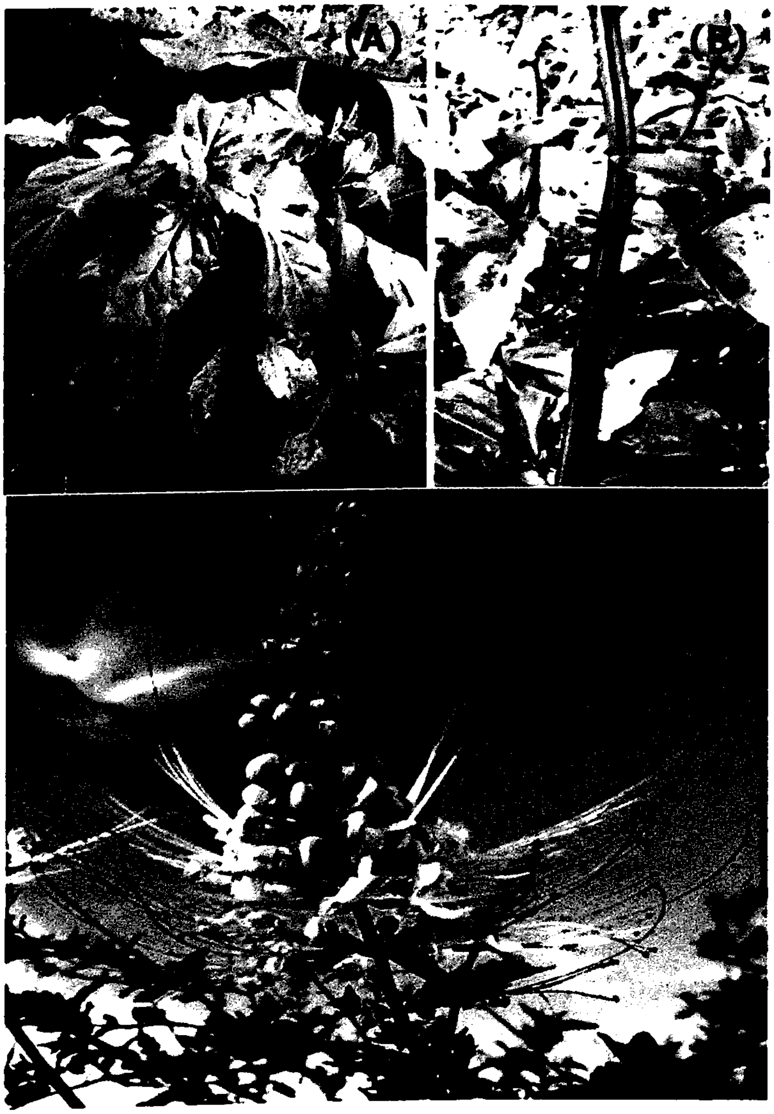

Figure 2.1 Diagram of *Orthosiphon stamineus* showing (A) leaves, (B) stem and (C) flower

2.3 Chemical Constituents and Phytochemical Content of *Orthosiphon stamineus*

The earliest phytochemical investigation on *Orthosiphon stamineus* started in 1989, when Guerin *et al.* (1989) isolated an oil, namely, methylripariochromene A from the plant. *Orthosiphon stamineus* looks similar to peppermint. However, the plant has a dry, salty, bitter taste. Chemically speaking, the plant is naturally bestowed with high amount of flavones, polyphenols, bioactive active proteins, glycosides, a volatile oil, and vast quantities of potassium.

Orthosiphon stamineus is famous for its flavonoids which are bioactive due to the presence of phenolic compounds in their structures. There were more than twenty phenolic compounds isolated from this plant, including lipophilic flavones, flavonol glycosides and caffeic acid derivatives such as rosmarinic acid and 2,3-dicaffeoyltartaric acid. They were identified and quantified by HPLC (Sumaryono *et al.*, 1991). According to Akowuah *et al.* (2004), a rapid, quantitative and simultaneous HPLC-based determination of major phytochemicals from the extract of *O. stamineus* leaves was reported and reconfirmed for its strong antioxidant potency and total phenolic content.

Besides, Akowuah *et al.* (2004) and Loon *et al.* (2005) also reported the presence of three methoxylated flavones, namely, sinensetin, eupatorin and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone from the methanolic leaves extracts of *O. stamineus* of Malaysia. The presence of rosmarinic acid from the leaves had also been reported by Akowuah *et al.* (2004).

2.3.1 Phenolic compound

Phenolic phytochemicals which are widely distributed in plant kingdom are the largest category of phytochemicals with antioxidant properties found in plants. These bioactive compounds inhibit lipid auto-oxidation by acting as radical scavengers and act as essential antioxidants that protect the propagation of the oxidative chain. Flavonoids, phenolic acids, and polyphenols are the three most important groups of dietary phenolics. Phenolics are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C_6H_5OH) is considered as the simplest class of this group of natural compounds while phenolic compounds are a large and complex group of chemical constituents that are available on plants (Walton *et al.*, 2003).

Among all of the chemically active constituents, polyphenols are the most dominant constituents in the leaf of *O. stamineus*, which has been reported to be effective in reducing oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems (Hollman and Katan, 1999). Phenolic compounds such as lipophilic flavones, caffeic acid derivatives (rosmarinic acid and 2,3-dicaffeoyltartaric acid), eupatorine, cichoric acid, sinensetin and methoxy flavones were found in *O. stamineus* (Olah *et al.*, 2004). Three highly oxygenated 2, 3-secoisopimarane-type diterpenes, named secoorthosiphols A, B, and C, have been isolated from the aerial parts of this plant (Awale *et al.*, 2002). According to a study of Huang and Zheng (2006), rosmarinic acid showed several bioactivities including anti-bacterial, anti-inflammatory and anti-carcinogenic activities.

2.3.2 Flavonoid compound

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which are usually very effective antioxidants (Hossain and Ismail, 2005). They are secondary metabolites in plants that do not have direct involvement with growth of plants.

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. They are synthesized by phenylpropanoid pathway. Available reports tend to show that secondary metabolites of phenolic nature including flavonoids are responsible for the variety of pharmacological activities including antimicrobial, antibacterial, anti-inflammatory, anti-allergic and antithrombotic actions (Mahomoodally *et al.*, 2005; Pandey, 2007). Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants as a response to microbial infection (Dixon *et al.*, 1983). Their activities are structure dependent. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations as well as degree of polymerization (Kelly *et al.*, 2002). Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals or by chelating metal ions (Kumar and Pandey, 2013).

The most prominent flavonoid compounds that can be isolated or extracted from *O. stamineus* are sinensetin, eupatorin, 5-hydro-6,7,3′,4′-tetramethoxyflvone, salvigenin, 6-hydroxy-5,7,4′-trimethoxyflavone, 5,6,7,3′-tetramethoxyflavone, ladanein, vomifoliol and so on (Hossain and Rahman, 2011). Among the flavonoid compounds that can be found in *O. stamineus*, sinensetin is the most common and important element to be identified. Sinensetin helps to relax the muscles of the walls of the internal vessels thus facilitating easier flow of urine and even the small particles that become stones. Besides, sinensetin was reported to have high chemo synthesizing effect which was used for the synthesis of the multi-drug resistance cell for anti-cancer drugs (Ahmad *et al.*, 2008).

2.4 Pharmacological Study on *Orthosiphon stamineus*

A lot of studies had proven that *O. stamineus* has a wide range of pharmacological properties that are beneficial for human health. Below are a few common ethnopharmacological studies on the specific uses of *O. stamineus.*

Previously, studies had been conducted on the anti-inflammation and analgesic activity of a standardised 50% methanol extract of *O. stamineus*. Researchers chemically induced edema in the hind paws of rats. The animals were then administered with the extract of *O. stamineus*. The extract significantly reduced the edema 3 and 5 hours after the swelling was induced. Besides preventing inflammation, *O. stamineus* also exhibited significant painkilling activity. Accordingly, Yam *et al.* (2008) provided supportive evidence that *O. stamineus* has as anti-inflammatory as well as non-narcotic analgesic agent. With these properties, the traditional medicinal uses of *O. stamineus* plant to cure pain and inflammation are justified.

Besides, *O. stamineus* also possesses anti-angiogenesis property which is one of the targets for cancer treatments (Beaux *et al.*, 1999) that are included in conventional chemotheraphy programs. Through the investigation of Sahib *et al.* (2009) on the anti-angiogenic activity of different extracts obtained from Malaysian *O. stamineus,* the methanolic extract of *O. stamineus* possessed the highest anti-angiogenic activity in rat aortic assay followed by the chloroform, petroleum ether and water extracts, descendingly. Further studies revealed that the extract has remarkable inhibitory activity on angiogenesis by blocking vascular endothelial growth factor (VEGF) signaling pathway. It was suggested that the anti-angiogenic effect is due to the extract's antioxidant potency.

Several previous studies had provided a scientific evidence for the traditional use of *O. stamineus* in the treatment of kidney stones and gout. *Orthosiphon stamineus* appears to enhance the activity of adenosine A receptor antagonists, and in turn stimulate the kidney for excessive flow of urine and thus sodium and other ions excretion. Besides, there was also another study reported that *O. stamineus* has the ability to reduce levels of uric acid in rodents (Arafat *et al.*, 2008).

2.5 Antioxidant Assay

Antioxidants are chemicals (both naturally occurring and man-made) that can prevent or slow cell damage. Antioxidants protect the body from damage caused by harmful molecules called free radicals. Any compound that can donate electrons and counteract free radicals has antioxidant properties. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals as well as by acting as oxygen scavengers (Shahidi and Wanasundara, 1992). Plants including herbs contain various phytochemicals which are rich sources of antioxidants and they provide defense mechanisms to plants against infection of hazardous organisms (Deans and Ritchie, 1987; Witkowska *et al.*, 2013).

Several herbs contain antioxidant compounds which protect the cells against the damaging effects of reactive oxygen species (ROS) (Narayanaswamy and Balakrishnan, 2011). ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide play a key role in the growth of various diseases (Halliwell and Gutteridge, 1990). Antioxidants from plant materials terminate the action of free radicals and protect the body from various diseases (Lai *et al.*, 2001). There is a growing interest all over the world for discovering the unexploited reservoir of medicinal plants.

A number of methods are available for the determination of free radical scavenging activity, but the assay employing the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) has received the maximum attention due to its ease of use and its convenience (Sanchez-Moreno *et al.*, 1998). DPPH is a stable free radical with purple colour, in which it will be reduced to a, a-diphenyl-ß-picrylhydrazine of yellow colour when reacting with an antioxidant agent, which is measured at 517 nm spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al.*, 2002).

The therapeutic benefit of medicinal plants is usually contributed to their antioxidant properties (Dixon *et al.*, 2005). Phenolic compounds possess diverse biological activities such as anti-inflammatory, anticarcinogenic and antiatherosclerotic

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