EFFECT OF ORGANIC SOIL AMENDMENTS ON GROWTH AND PHYTOCHEMICAL CONTENT OF SABAH SNAKE GRASS (*Clinacanthus nutans*)

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURAL SCIENCE WITH HONOURS

CROP PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2016

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: EFFECT OF ORGANIC SOIL AMENDMENTS ON THE GROWTH AND PHYTOCHEMICAL CONTENT OF SABAH SNAKE GRASS (*Clinacanthus nutans*)

IJAZAH: DEGREE OF BACHELOR OF AGRICULTURAL SCIENCE WITH HONOURS

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ACKNOWLEDGEMENT

First and foremost I would like to take this opportunity to express my outmost appreciation to my dedicated supervisor, Madam Devina David. I would like to thank her for giving me the opportunity to gain rich experiences and knowledge in conducting my final year project. I would like to thank her for the productive discussions, valuable teaching on the project planning and helpful suggestions for my final year project. I am truly grateful for her patience, encouragement, advice, support and effort in guiding me to accomplish my dissertation. I am glad and lucky to have her as my supervisor who cared so much about my final year project.

I also wish to express my appreciation to the lab assistants, Mrs. Nurul Syakina Marli, Mr. Rohizan Basir and the field laboratory staffs, Mr. Federick, Madam Santy and Miss Siti who guided, helped and gave their best to provide me materials and equipment needed for my project.

Besides, I would like to offer my special thanks to my friends and course mates who have assisted me during the preparation of this proposal and the beginning of my project.

Last but not least, I would like to dedicate my heartfelt thanks to my beloved and wonderful family members for their cares, love, support and encouragement. They are my motivation; they love and support made me kept pushing me for the best.

Again, I would like to dedicate special thanks to all the people who helped me directly or indirectly during this period. This final year project would not be a success without the support of all these peoples.

ABSTRACT

Clinacanthus nutans is one of the traditional herbs, which is famous for its multiple medicinal usages and well known for its antioxidant ability. The objective of this study was to study the effect of organic soil amendments in enhancing the growth and phytochemical contents of *C. nutans*. These organic soil amendments were on-farm resources, such as Empty Fruit Bunch (EFB), Spent Mushroom Substrate (SMS), Rice Husk Biochar and Rice Bran. Topsoil was used as control medium in this study while all the soil organic amendments were applied in topsoil medium in ratio of 1:20 wt/wt (100g organic soil amendment with 2000g topsoil). After 13 weeks of planting, it was found that rice bran treatment enhanced the most in *C. nutans* growth development (plant height, leaf and branch number, leaf width and leaf dry matter content) followed by rice husk, EFB, SMS and control treatment. Extract of *C. nutans* treated with rice bran also showed the highest reducing power in DPPH scavenging activity, followed by EFB and SMS treatment. Meanwhile, *C. nutans* treated with EFB reported highest total phenolic content among all treatments. However, the total flavonoid content between control and organic soil amendment has no significant different. This study concluded that rice bran treatment is the best treatment in promoting growth, yield and antioxidant activity of *C. nutans.* While EFB treatment is the most ideal treatment for enhancing total phenolic content in *C. nutans.* All data were analyzed statically by using one-way ANOVA test with 5% significant level.

KESAN BAHAN PEMBAIK TANAH ORGANIK TERHADAP PERTUMBUHAN DAN KANDUNGAN FITOKIMIA BELALAI GAJAH (*Clinacanthus nutans*)

ABSTRAK

Belalai gajah adalah salah satu herbal tradisional yang terkenal dalam kalangan masyarakat kerana ia mampu menyembuh pelbagai penyakit. Objektif bagi melakukan eksperimen ini adalah untuk menkaji kesan bahan pembaik tanah organik terhadap pertumbuhan dan kandungan fitokimia Belalai gajah. Bahan-bahan pembaik tanah yang digunakan dalam eskperiment ini adalah tandan buah kosong kelapa sawit (EFB), sisa penanaman cendawan (SMS), beras sekam biochar dan deddak padi. Tanah atas digunakan sebagai rawatan kawalan dan kadar aplikasi bagi semua bahan-bahan pembaik tanah adalah 1:20 (100g bahan pembaik tanah dengan 2000g tanah atas). Selepas 13 minggu, ia didapati rawatan deddak padi memberi kesan paling siknifikasi terhadap penanaman Belalai gajah dengan rekod yang paling tinggi dalam ketinggian, bilangan daun and dahan, panjang dan kelebaran daun dan kandungan bahan kering dalam daun Belalai gajah; diikuti dengan rawatan beras sekam biochar, tandan buah kosong kelapa sawit (EFB), sisa penanaman cendawan (SMS) dan rawatan kawalan. Tambahan pula, ekstrak Belalai gajah daripada rawatan deddak padi juga menunjukkan kesan paling siknifikasi dalam activity antioksidan dengan banyak mengurangkan radikal bebas dalam ujian DPPH; diikuti dengan ekstrak Belalai gajah daripada rawatan tandan buah kosong kelapa sawit (EFB) dan rawatan sisa penanaman cendawan (SMS). Manakala Belalai gajah yang ditanam dengan rawatan EFB menunjukkan kandungan jumlah fenolik yang paling tinggi banding dengan rawatan-rawatan lain. Tetapi, kandungan jumlah flavonoid tidak ada perbezaan yang siknifikasi dalam semua rawatan. Kesimpulanya, kajian ini menunjukkan rawatn deddak padi dapat meninggikan pertumbuhan, hasil dan aktiviti antioksidan Belalai gajah. Manakala rawatan EFB adalah rawatan yang berkesan dalam penambahan kandungan jumlah fenolik Belalai gajah. Semua unsur dalam kajian ini termasuk unsur pertumbuhan, jumlah kandungan fitokimia dan aktiviti antioksidan akan dikira dengan menggunakan satu hala ANOVA pada aras keertian 5%.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

µg	Micro gram
°C	Degree Celsius
mg	Milligram
ml	Milliliter
nm	Nanometer
mm	Millimeter
M	Molar
m^2	Meter square
m^3	Cubic meter
ppm	Parts per million
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
H_a	Alternative Hypothesis
H_0	Null Hypothesis
EFB	Empty Fruit Bunch
SMS	Spent Mushroom Substrate
CHS	Enzyme Chalcone Synthase
GAE	Gallic acid equivalent
QE	Quercetin equivalent
FSA	Faculty of Sustainable Agriculture
ANOVA	Analysis of Variance
SPSS®	Registered Statistical Package for the Social Sciences

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Background

Throughout the ages, humans have relied on nature for their basic needs for the production of food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours and fragrances, and not the least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Before the rise of chemical medicine like Panadol (Paracetamol), human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1972). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Benny, 2000) although there are more and more people rely on Western medicine and treatments.

Recently, Malaysia government has highlighted several core strategies for the country's economic growth in order to achieve the standard of a developed country by 2020. This includes the introduction High-Value Herbal Products under Entry Point Project (EPP1) for the nation's Agriculture NKEA (National Key Economic Areas) of Economic Transformation Programme (ETP). In this project, government has commercialized on 11 types of herbs, and one of it is Sabah Snake Grass (*Clinacanthus nutans*). This shows the determinant of government in developing the traditional herbs and introducing them to worldwide, not only in bringing up our country's economy but also to introduce their important medicinal values to all over the world, saving more life.

Sabah snake grass (*C. nutans*) is an old traditional herb. It is originally found and grown in tropical Asia and utilized in Malaysia due to its' multiple medicinal usage (Ali *et al.*, 2014). In addition, it is also known as Belalai Gajah in Malay and can be found easily in East Malaysia, Sabah (Roosita *et al.*, 2008). It has been used in folk medicine to cure various kinds of ailments such as snake bites, kidney failure and cancer (Kameh *et al.*, 2013). The phytochemical content is the chemicals content in a plant. It is categories into two constituents, primary and secondary constituents. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.*, 2007). Few studies are conducted before on the phytochemical analysis of *Clinacanthus nutans*; it contains phytochemical compounds like alkaloids, phenolic acids, tannins, flavonoids, cardiac glycosides, saponins, diterpenes and phytosterols (Sathisha, 2013; Ho *et al.*, 2013).

Soil amendment, also named as soil conditioner; is a type of product which functions in increasing soil properties in physically, chemically and biologically. According to British Columbia Environmental Farm Planning which set up in year 2010; soil amendment is the use of on-farm and off-farm source to help in increasing properties and fertility of soil. For on-farm source, examples are like compost, crop residue, manure, contaminated runoff, silage juice, spoiled feed, wash water, spent soilless media, spent mushroom media, spent nutrient solution empty fruit bunch, rice husk char and rice bran. Mostly, all these products are organic and farm wastage. Besides, there were also studies on effect of rice husk biochar on growth of lettuce (*Lactuca sativa*) and Chinese cabbage (*Bassica chinensis*) (Sarah *et al.*, 2013) and the effect of rice husk and wood biochar on growth of water spinach (*Ipomoea aquatica*) (Varela *et al.*, 2013).

1.2 Justification

The reason for carrying out this experiment is to identify the role of soil amendments on the growth and also on phytochemical content of *C. nutans*. By using different types of organic soil amendments on planting the *C. nutans*, the growth and phytochemical contents of crops maybe vary during growth period. This study also can provide valuable information on the capability of various organic soil amendments on *C. nutans* growth parameters and changes in phytochemical contents. Some experiments were done before on the effect of organic soil amendments like empty fruit bunches, wood ad rice husks on growth of sweet corn, where it is proved that these soil amendments are effective on stimulating the growth (Huda *et al.*, 2014).

Hence, by conducting this experiment, it can be a stepping stone for further and detail studies on the changes of *C. nutans* growth and phytochemical contents. Moreover, by using organic amendments, it is more sustainable and the same time, we are practicing the 3R practices: reduce in using synthetic materials, recycle the unwanted on-farm and off-farm residues and reuse them in the soil.

1.3 Objectives

a) To evaluate the effect of soil amendments on growth and yield of Sabah snake grass (*Clinacanthus nutans*).

b) To evaluate the effect of soil amendments on phytochemical content of Sabah snake grass (*Clinacanthus nutans*).

1.4 Hypothesis

H_0: There is no significant effect on the types of soil amendments for growth and yield of *Clinacanthus nutans.*

H_a: There is a significant effect on the types of soil amendments for growth and yield of *Clinacanthus nutans.*

H_0: There is no significant difference on the effect of different types of soil amendments for phytochemical content of *Clinacanthus nutans.*

H_a: There is significant difference on the effect of different types of soil amendments for phytochemical content of *Clinacanthus nutans.*

CHAPTER 2

LITERATURE REVIEW

2.1 Sabah Snake Grass (*Clinacanthus nutans*)

Acanthus is a member of the Acanthaceae family which comprises of 250 genera and about 2500 species and it is also a taxon of dicotyledonous flowering plants. This family mostly is tropical herbs; shrub and some of them are epiphytes. At family level, they can be easily recognized morphologically by their simple, opposite, decussate, entire leaves, zygomorph flowers and their superior ovary (Ng, 2013). *Clinacanthus nutans* has various common names. It is known as Dandang gendis (Indonesia), Belalai gajah (Peninsular Malaysia), Phaya yor (Thailand), You Dun Cao (China), Sha Ba She Cao (Sabah, Malaysia). The common English name is Snake plant or snake grass (Ng, 2013).

2.1.1 Botanical Description

Clinacanthus nutans (*C. nutans*) is a shrub or perennial herbs that can grow up to 1m in height with pubescent branches. The stem is torete, striate and glabrescent. The leaves are simple, opposite, narrowly elliptic-oblong or lanceolate (2.5 cm-13 cm long x 0.5 cm-1.5 cm wide) (Panyakom, 2006). The leaves have apex acute or acuminate and exsculptate; dentate or subentine margins. Both surfaces of leaves are pubescent when young then glabrescent. The leaf base are cuncate, obtuse rounded or truncate; often oblique (Ng, 2013). While for its petiole, it is around 3-15 mm long (Deng *et al.*, 2011). According to Panyakom (2006), the flowers are sordidly yellow or greenish yellow. They are in dense cymes at the top of branches and branchlets; always covered with 5-alpha cymules. The calyx of flower about 1cm long with grandular-pubescent. Its corolla is dull red with green base, about 3 cm-4.2 cm. The stamens are exserted from the throat of corolla. The ovary is compressed into two cells and each cell having two ovules. The styles are filiform with shortly bidentate. The capsule is oblong basally wrapped into 4-seeded short stalk (Ng, 2013). Table 2.0 described the taxonomy of *Clinacanthus nutans.*

Table 2.1 Taxonomy description of *Clinacanthus nutans*

Taxonomy	**Description**
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Acanthaceae
Genus	*Clinacanthus*
Species	nutans-Lindau
Scientific name	*Clinacanthus nutans Lindau*

Source: Ng, 2013.

2.1.2 Medicinal Values of *Clinacanthus nutans*

Clinacanthus nutans plays an important role in traditional herbal medicine for treating skin rashes, insects and snake bites, lesions caused by herpes simplex virus, diabetes mellitus, fever and diuretics (Tuntiwachwuttikul *et al.*, 2004). On the other hand, scientists in Thailand do proved that it can treat dysentery and fever. Due to its anti-cell lysis property, this plant has been used as anti-venom for snake and scorpion bites and also removes nettle rashes (Afiq, 2011). While in China, this plant is used to treat inflammation such as hematoma, bruises on eye, anxieties, injuries and rheumatism (Senny, 2009). In Indonesia, Sabah snake grass is used to treat diabetic patients, dysuria and dysentery by consuming the decoction from fresh leaves boiled with hot water (Afiq, 2011).

This plant's natural minerals help in adapting normal menstrual function, relieving pain, anaemia, repairing of fractured bones and jaundice. Despite of that, people believe *C. nutans* can cure cancer disease due to the flavonoid content. The flavonoid in plants acts as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening (Pietta, 1999). Mostly, people are more interest in the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 1999). However, it does not have any scientific research to prove that it can kill the cancer cell and cure the disease. The medicinal value of this herb is focused on its leaves where it can consume as raw material or mixed with other juices such as apple juice, sugarcane or green tea and served as fresh drink (Muhammad *et al.*, 2014).

2.1.3 Economic Importance of *Clinacanthus nutans*

Clinacanthus nutans has great contribution in agricultural sector due to its high value herbal products like tea bags, medicinal cream, lotion, drug and secondary metabolites products. Apart from that, an extracts from leaves can be used as cream and drinks for disease treatment as well. The dried leaves are extracted with alcohol as cream and serves as medicine in relieve pain and healing herpes infection (Sangkitpporn *et al.*, 1993). Hence, it has been selected as one of the most potential herbs under the first Entry Point Project (EPP1). The government believed in the potential of the herbs in propelling the sustainability of the nation's Bio-economy sector. By developing the herbal industry alongside the latest global development, the trade value of the herbs sector is estimated to rise over RM2 trillion by year 2020 (Daily Express, 2013). The estimated value is a threefold increase compared to the RM777 billion worth of trade in the herb sector in 2009. On the local front, the Ministry Malaysia Agricultural Department estimated the herb market will expand by 15% a year from RM7 billion in 2010 to around RM29 billion by year 2020.

2.2 Organic Soil Amendments

Soil amendment is the material that added to a soil to provide nutrients for crop growth and to provide materials for soil physical improvement such as increase in water retention, permeability, water infiltration, drainage, aeration and structure. The goal is to provide a better environment for roots. However, misusing of soil amendments will result not only in damage to crops but also can cause negative impacts on it like stunt growth and reduction in yield production. Hence, an amendment must be thoroughly mixed into the soil. If it is merely buried, its effectiveness is reduced, and it will interfere with water and air movement and root growth. The soil amendment can broadly divide into organic and inorganic soil amendments. The organic amendments come from something that is alive. While for inorganic amendments, are either mined or man-made. Organic amendments include sphagnum peat, wood chips, grass clippings, straw, compost, manure, biosolids, sawdust and wood ash. Inorganic amendments include vermiculite, perlite, tire chunks, pea gravel and sand. The organic soil amendment is mainly made up of plant and animal by-products (Davis and Whiting, 2014).

The plant based soil amendments are like alfalfa meal, cottonseed meal, fruit pomaces, leaf compost and wood ash. All these soil amendments were on-farm sources and organic. According to the British Columbia Environmental Farm Planning Workbook (2010), wood ash contains about 2% phosphates and 6% potash; alfalfa meal contains around 3% nitrogen and is commonly used as an animal fed. While for cottonseed meal and soybean meal, they are rich source of nitrogen (7%) (Bart, 1998). For animal based soil amendments, they are made up of animal waste products like blood, bone and feather. Compare to plant based soil amendments, the nitrogen contain of these animal based soil amendments are fairly high (7%-10%) (Bart, 1998).

2.2.1 Spent Mushroom Substrate (SMS)

Spent mushroom substrate (SMS) is the remaining composted organic material after the mushroom planting is harvested. Mushrooms are grown in a mixture of nature products, like horse-bedded straw, hay, poultry manure, ground corn cobs, cottonseed hulls and other substances. These mixtures are composted in piles or ricks, forming a dark brown, fibrous and liable organic growing media. The media is brought into mushroom houses after completing composting process, where it will be placed into trays and used as substrate for growing mushroom. After harvesting the mushroom, these residues will remove from the houses and pasteurized with steam to kill insects, pathogens, and mushroom remnants (Peter and Andrew, 2015).

The SMS function in improving the structure of clay soils, reduce surface crusting and compaction, promote drainage, increase microbial activity, and provide nutrients to crops. The pH of SMS is range between 6.0-8.0; containing around 1.5-3% of nitrogen (N) on a dry weight basis, 0.5-2% of phosphorus (P), 1-3% of potassium (K), 3-6% of calcium (Ca) and 0.4-1% of magnesium (Mg) (Peter and Andrew, 2015).

2.2.2 Empty Fruit Bunch (EFB)

Empty fruit bunch (EFB) is the by-product from crude palm oil mill. It is normally used as mulches in crops planting to protect the soil surface and conserve soil water and nutrients (Teh *et al.*, 2010). EFB also consists of high water holding capacity (Farahzety and Aishah, 2013). Table 2.2 shows the chemical composition and properties of empty fruit bunch.

Table 2.2 Chemical composition and properties of empty fruit bunch (EFB)

Composition	Value
pH	7.93
Nitrogen (N)	1.46%
Carbon (C)	12.81%
Phosphorus (P)	1.47%
Potassium (K)	2.58%
Calcium (Ca)	1.69%
Magnesium (Mg)	0.24%
Copper (Cu)	89.2%
Iron (Fe)	11800ppm
Manganese (Mn)	432.2ppm
Zinc (Zn)	249.3ppm

Source: Farahzety and Aishah, 2013.

2.2.3 Rice Bran

Rice bran is the hard outer layers of rice grain, consisting combination of aleurone and pericarp. It is the by-product of milling in the production of refined grains. Rice bran is rich in dietary fiber, essential fatty acids and contains significant quantities of starch, protein, vitamins, dietary minerals and phytic acid, which is an antinutrient that prevents nutrient absorption (Kahlon *et al.*, 1990). It also been recognized as an excellent source of vitamins and minerals, it has been under-utilized as a human food and has traditionally been used primarily in animal foods. Research conducted in last two decades has shown that it contains a unique complex of naturally occurring antioxidant compounds (Moldenhauer *et al.*, 2003). Table 2.3 shows some of the composition of rice bran.

Table 2.3 Chemical composition and properties of rice bran

Composition	Value
Total Dietary Fiber	20.9%
Soluble Dietary Fiber	2.9%
Fat	22.4%
Nitrogen (N)	2.4%
Carbon (C)	14%
Phosphorus (P)	56.00mg/kg
Potassium (K)	7.93%
Calcium (Ca)	0.12%
Magnesium (Mg)	1.80%

Source: Kahlon *et al.*, 1990; Moyin-Jesu, 2015.

2.2.4 Rice Husk Biochar

Rice husk is the outermost of the paddy grain that is separated from the rice grains during the milling process. It is also the protection layer of paddy grain, yellowish in colour and has a convex shape. The size is slightly larger than a grain of rice, up to 7mm in length. Besides, it is lightweight, having a ground bulk density range between 340 kg/m^3 and 400 kg/m^3 (Alaric, 2013). The rice husk will burn through ferro-cement furnace at temperature 600 ℃ for 3 hour (Ahmad *et al.*, 1997). This will result the best characterization of ash content, fixed carbon, and moisture content (Otaru *et al.*, 2013). It can significantly improve soil properties by decreasing soil bulk density, enhancing soil pH, adding organic carbon, increasing available nutrients and removing heavy metal from cropping system; indirectly, it will increase in yield production (Williams *et al.*, 1972). The chemical composition of rice husk biochar is listed in Table 2.4.

Table 2.4 Chemical composition and properties of rice husk biochar

Composition	Value
Silicon oxide (SiO_2)	88.32
Aluminium oxide (Al_2O_3)	0.46
Iron (III) oxide (Fe_2O_3)	0.67
Calcium oxide (Cao)	0.67
Magnesium oxide (MgO)	0.44
Disodium trioxide (Na_2O_3)	0.12
Potassium oxide (K_2O_3)	2.91%

Source: Habeeb and Mahmud, 2010.

2.3 Effect of Organic Soil Amendments on Plant Growth

According to British-Columbia Environmental Farm Planning (2010), the primary role of soil amendments is to provide nutrients for crop growth and to provide materials for soil improvement. It can improve the physical properties of soil like soil moisture content, water holding capacity and hydraulic conductivity (Ahmad *et al.*, 2014; Belyaeva and Haynes, 2012; Brewer *et al.*, 2012; Chan *et al.*, 2007; Chen *et al.*, 2010; Ventura *et al.*, 2012). Few studies were conducted to test the effect of organic soil amendments on crops growth like sweet corn (Huda *et al.*, 2014), water spinach (Varela *et al.*, 2013), lettuce and cabbage (Sarah *et al.*, 2013) and tomato (Chang and Yau, 1981; Iwase *et al.*, 2000). In these experiments, soil amendments like wood biochar (WB), rice husk biochar (RHB), empty fruit bunch (EFB) and spent mushroom substrate (SMS) were used to test their efficiency on crops growth. Application of 15 and 30 tonnes per hectare of empty fruit bunch and wood biochar promoted the most growth of sweet corn shoot dry weight. While for corn height, 30 tonnes per hectare of empty fruit bunch and wood biochar enhance the best growth performance (Huda *et al.*, 2014).

Rice husk biochar improved biomass production, plant weight, stem size and leaf length while the wood biochar increased the plant height of crops by increasing root size and leaf width (Varela *et al.*, 2013). Application of spent mushroom substrate has increased 7 fold of yield production of tomatoes compare to others crops (Chang and Yau, 1981; Iwase *et al.*, 2000). Earlier research showed that the application of spent mushroom substrate also improved the moisture holding capacity of soil and resulted in increased plant uptake nutrients (Harris, 1992).

However, some studies showed no beneficial effect (Devereux *et al.*, 2012) of soil amendments application as reported by Gajic and Koch (2012); Kloss *et al.*, (2014). The additional of biochar soil amendment caused high pH biochar into high soil pH, causing problems like stunt growth (Van Zwieten *et al.*, 2010; Haefele *et al.*, 2011).

2.4 Phytochemical Constituents and Antioxidant activity in *Clinacanthus nutans*

A study by Ho *et al.*, (2013), revealed phytochemicals like saponins (foam test), phenolic compounds (Iron III Chloride test), diterpenes (copper acetate test) and phytosterols (Liberman Buchard's test and Salkowski's test) were presence while alkaloids (Mayer's reagent test), tannins (gelatin test) and flavonoids (alkaline reagent test and lead acetate test) showed absence result in methanolic extract of *C. nutans* (Ho *et al.*, 2013)

2.4.1 Total Phenolic Content in *Clinacanthus nutans*

Phenolic compound is one of the important secondary metabolites and bioactive compounds of plant. Polyphenols are bioactive constituents present in food plants which are very important in the control and prevention of tissue damage by activated oxygen species due to their antioxidant effects (Rucker, 2004). Phenolic compounds also function to increase bile secretion, reduce blood cholesterol and lipid levels and inhibit microbial growth. Besides, phenolic compounds are crucial for plant growth and plant reproduction (Pengelly, 2004). Few studies were conducted in estimating total phenolic content in *C. nutans*. According to Tiew *et al.* (2014), the total phenolic content of the *C. nutans* leaf extracts was determined using Folin-Ciocalteu reagent with extract concentration of 10mg/ml. The sample was prepared by mixing 0.4ml of 10mg/ml of extract with 0.4 ml of Folin-Ciocalteu reagent (50% v/v) and 15% sodium carbonate. Samples were diluted with deionized water and undergo incubation. Gallic acid was used to plot standard calibration curve and absorbance of each sample was measured at 760 nm using UV-Vis Spectrophotometer. The total phenolic content of extracts was expressed as milligrams (mg) of gallic acid equivalents (GAE) per 1g of dry plant extract trichloride (Tiew *et al.*, 2014).

2.4.2 Total Flavonoid content in *Clinacanthus nutans*

Flavonoid is a sub-class of phenolic and known with its polyphenolic structures. Flavonoid plays important role in protecting biological systems against harmful effects of oxidative processes on macromolecules. Flavonoids are reported possess antiulcer activity, hepatoprotective activity, anti-inflammatory activity, antidiabetic effects,

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