CHAPTER II

LITERATURE REVIEW

2.1 The Plant

Clinacanthus nutans has different popular names. For example, it known as Dandang gendis in Indonesia, as Belalai gajah in Peninsular (Malaysia), as Phaya yor in Thailand, as You Dun Cao in China and as Sha Ba She Cao in Sabah (Malaysia). The common English term of it is snake grass or snake plant. In addition Snake grass has many synonyms. For instance, *Clinacanthus siamensis, Clinacanthus spirei, Clinacanthus burmanni,* and *Clinacanthus angustus* (Nees, 1847). These species of *Clinacanthus* are traditionally utilized as herbs in in China and Southeast Asia.

Clinacanthus nutans belong to Acanthaceae family comprise of 250 genera and 2500 species. Acanthus is a member of it and this family is also a taxon of dicotyledonous flowering plants. Some of Acanthaceae family is epiphytes and commonly grew in tropical areas. This family can be simply identified morphologically by their simple, opposite, decussate, full leaves, zygomorph flowers and their larger fruit.

2.1.1 Botanical Description

The taxonomic classification and nomenclature of *Clinacanthus nutans* are showed in Table 2.1

Clinacanthus nutans can grow up to 1m in height with young branches and it is a shrub or perennial herbs. Its leaves as shows in the Figure 2.1 are opposite, simple, narrowly elliptic-oblong or lanceolate (2.5- 13 cm long x 0.5- 1.5 cm wide), whereas, the stem is torete, striate and glabrescent (Santi et al., 2006).

Table 2.1: The taxonomic classification of *Clinacanthus nutans*

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

Hu & Daniel (2011) pointed that the leaves possess apex acute or acuminate and exsculptate; dentate or subentine limits. When the leaves are young, both its surfaces are pubescent, after that became glabrescent. The leaf base is cuncate, round or shortens, often oblique and the petiole is 3-15mm long.

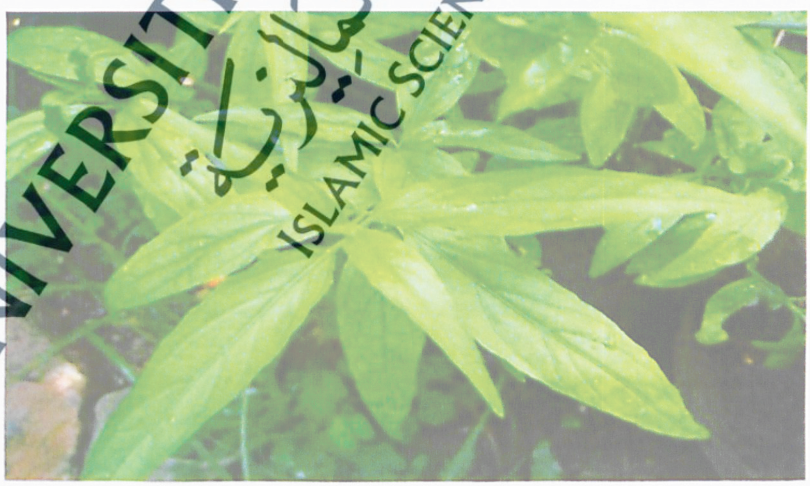

Figure 2.1: The snake grass (*Clinacanthus nutans*)

Panyakom (2006) explained that the *Clinacanthus nutans* flowers are sordidly yellow or greenish yellow in color. They are compact cymes at the upper of branches and branchlets covered with 5-alpha cymules (Backer et al., 1965).

2.1.2 Bioactive Compounds Identified in *Clinacanthus nutans*

A number of bioactive substances that include belutin (Lin et al., 1983), stigmasterol, lupeol, *b*-sitosterol (Dampawan et al., 1977), C-glycosyl, isovitexin, flavones, shaftoside, vitaxin, isomollupentin-7-O-β-glucopyranoside, isoorientin, orientin (Teshima et al., 1997), sulfur-inclosing glycosides, cerebrosides (Teshima et al., 1998), two glycoglycerolipids (Satakhun,2001), a monoacyl-monogalactosylglycerol (Tuntiwachwuttikul et al., 2004), 13^2-hydroxy-(13^2-R)-phaeophytin b, 13^2-hydroxy-(13^2-S)-phaeophytin a and 13^2- hydroxy-(13^2-R)-phaeophytin (Sakdarat et al., 2009) were discovered in the leaves of *Clinacanthus nutans* when diverse solvent systems were applied. The two glycoglycerolipids have been stated to show antiviral activity (Sakdarat et al. 2009).

Flavonoids components possess an extensive array of biological activity, which are abundantly originated in plant kingdom and have ability to protect the plant from UV radiations and fungal contamination. Flavonoids have several medical properties such as neuroprotective, anti-bacterial, anti-inflammatory, anti-viral, and anti-allergic (Sandhar et al., 2011). Vitexin, shaftoside, isovitexin, isoorientin, orientin, are identified in *Clinacanthus nutans* extract (Teshima et al., 1998). A study on flavonoids portion can lead to the probable applicants in drug innovation.

Cytokines are immunomodulating mediators retaining both positive and negative regulatory impacts on numerous target cells. They can be anti-inflammatory or pro-inflammatory (Opal & Depalo, 2000). It was revealed that *Clinacanthus nutans* plant extract showed anti-inflammatory effects by activating the lymphocyte proliferation and interleukin-4 (IL-4) creation (Sriwanthana, 1996).

2.1.3 Medicinal Uses of *Clinacanthus nutans*

Clinacanthus nutans is a significant therapeutic plant. It affords several healing usages and products. Many materials such as lotion, cream, drug, tea, and secondary metabloites products can be produced from that plant. Additionally, leaves extracts, which is performed using alcohol can be used as cream and drinks to treat some diseases and relieve pains caused by herbs infection (Sangkitporn et al., 1993).

Medicinal effects of *Clinacanthus nutans* have concerned pharmacologist interest to explore its medicinal properties. Many researches showed *Clinacanthus nutans* therapeutic potential (Direkbusarakom et al., 1998; Kongkaew & Chaiyakunapruk, 2011; Kunsorn et al., 2013; Lau et al., 2014; Pannangpetch et al., 2007; Sookmai et al., 2011; Wanikiat et al., 2008; Yong et al., 2013a). According to those researches some of the *Clinacanthus nutans* pharmacological properties are found as follows:

2.1.3.1 Anti-Viral

The anti-viral activities of *Clinacanthus nutans* included Herpes Simplex Virus (HSV) (Kunsorn et al., 2013), Human Papillomavirus (HPV) (Sookmai et al., 2011) and Yellow-head rhabdovirus (YRV) (Direkbusarakom et al., 1998).

2.1.3.2 Anti-Oxidant

Clinacanthus nutans showed antioxidant activities as react with Ferric Reducing Antioxidant Power (FRAP), free radical-induced hemolysis protection, 2, 2 -azobis (2-amidino-propane) dihydrochloride (AAPH), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Pannangpetch et al., 2007) and Galvinoxyl radicals (Yong et al., 2013a).

2.1.3.3 Anti-Inflammatory

The extract of *Clinacanthus nutans* has displayed anti-inflammatory effects by producing the lymphocyte proliferation and interleukin-4 (IL-4) production (Sriwanthana, 1996), in addition to neutrophil responsiveness inhibition (Wanikiat et al., 2008).

2.1.3.4 Neuromodulator

The *Clinacanthus nutans* also had shown an ability to modulate cholinergic neurotransmission (Lau et al., 2014).

2.1.3.5 Anti-Cancer

Clinacanthus nutans plant extract reported to possess anticancer effects on human, which include antiproliferative effects on: gastric cancer cell line (SNU-I), colon adenocarcinoma cell line (LS-174T), Burkitt's lymphoma cell line (Raji), lungcancer cell line (NCI-H23), erythroleukemia cell line (K-562), liver hepatocellular carcinoma (HepG2) (Yong et al., 2013a) and cervical cancer cell line (HeLa).

2.2 Extraction by Solvents

Solvent extraction is a technique that can provide a good understanding about the chemicals in food materials. Thus, if must be taken into account to gain high purity of product, less contamination and high recovering substances from complex and dilute matrixes. This means, the selectivity of solvents should be carefully made to increase a good separation process.

Type of solvent is important factor in extraction. The differences in the chemical properties of solvents and the solute's chemical characteristics lead to differ their ability to extract substances. In addition, boiling point, surface tension, density, viscosity, flammability, corrosiveness, toxicity, compatibility, stability, cost and availability are other factors affecting the extraction efficiency (Cowan, 1999). Petroleum ether, Chloroform, methanol, water and hexane are the most used solvents for extraction of plant materials. Hexane, chloroform, methanol and distillated water have been used to extract *Clinacanthus nutans* compounds (P'ng et al., 2012; Sakdarat et al., 2009; Yong et al., 2013b; Yoosook, et al.,1999).

The extraction operation is to offer a maximum yield of the target chemical components in food materials, which have biological activities (Spigno et al., 2007). For instance, several studies have utilized different solvents to extract polyphenols from plant materials. It was observed that the concentration of such chemical substances depends on the solvent type and extraction system (Goli et al., 2005; Hayouni et al., 2007a; Pinelo et al., 2004). Moreover, it has been found that the extracting solvent significantly influenced the biological activities of such compounds (Turkmen et al., 2006). For example, methanol has been found as a good solvent to extract poluphenols (Dai & Mumper, 2010). Although, alcoholic solvents are not selective for phenols, they have been widely utilized to extract them from natural sources. That is because of their ability to give quite great yield of total extract (Spigno et al., 2007).

Solvent extraction as a pure or mixture with different polarities varies significantly in their extraction capability and selectivity for leaf phenolics and their antioxidant activities (Trabelsi et al., 2010). It is stated that water and organic solvents, which have been used combined or individually in a ratio of 90:9.5:0.5 (acetone; water; acetic acid) and a ratio of 60:30:10 (ethyl acetate; methanol; water) considerably influenced content of total polyphenol of *Quercus coccifera* L. and *Juniperus phoenicea* L fruit extracts (Hayouni et al., 2007b). A number of researchers mixed various kinds of solvents to obtain an enhanced solvent system in plant extraction procedure (Gurjar et al., 2012).

2.3 Evaporation Techniques

In extraction process, the solvent must be evaporated to concentrate the extract. The evaporation technique is performed in different ways; such techniques include stationary evaporation and rotational type evaporation. The rotational type evaporation is the most popular process utilized to evaporate a solvent from an extract (Nkambule, 2008).

2.3.1 Rotary Evaporator

Rotary evaporator (rotavapor) is an important evaporation unit combined with a rotating evaporation flask that is developed by Buchi. Vacuum evaporation is mostly used due to its effectiveness in removing the solvent. In addition, it is faster than the stationary evaporation flasks and also can produce a very effective heat transfer, at the same time, confirming fully mixing and avoiding overheating of the extract (Nkambule, 2008).

Rotation movement transfers a liquid sample as a thin film to the entire flask surface, noticeably increasing evaporation speed and helping transfer of heat from a heating source. Moreover, sealing system, which is rotating flask and vapor vessel are combined by, assists the operation to be under vacuum. Additional increasing the speed of the evaporation process due to a decrease in boiling point of the solvent and effective removal of the vapor phase. Also, vacuum operation allows heat-labile constituents to be effectively concentrated without degradation (Buchi., 2008).

2.4 Chromatography Technique

Chromatography technology is widely utilized for separation, isolation and purification. This technique was established in 1906 by a Russian botanist, Mikhail Tswett, who separated various color components from extract of crushed leaf by passing it through a column consisting alumina, sucrose and calcium carbonate. Consequently, it was called *chroma graphein,* which is *a* Greek word, where *chroma* means "color" and *graphein* means "to write" (Pedersen & Myers, 2010; Wixom, 2001). It is a physical process of separation where, the separated components are distributed between two phases. Those two phases are mobile phase and stationary phase (Ahuja, 2003). Silica, alumina or C-18 powders are the most used stationary phases, whereas the mobile phase can be gas or liquid. The mobile phase carries the analyte through the stationary phase. The analytes ideally equilibrate or differentially partition among the two phases, leading to a diverse migration rate through the system. Two type of chromatography are liquid chromatography and gas chromatography (Villas-Boas et al., 2007). The phytoconstituent measurement is typically attained by gas (GC) and liquid chromatography (LC) linked with specific detection schemes (Eisenhauer et al., 2009).

2.4.1 Gas Chromatography

The principle of gas chromatography, is to separate compounds according to their polarity difference or boiling points in moving gas. That means a mixture of compounds is separated based on their affinity of interaction with the stationary phase filled into the capillary column of gas chromatography. Gas chromatography is very sensitive and offers quantitative and qualitative results in a single process (Harborne, 1998).

2.4.2 Mass Spectroscopy

Mass spectroscopy (MS) is also a detector used in chromatography technique, since it provides important information about the molecular weight and mass fragmentation pattern in explaining a structure of a compound. It can be used to analyze different types of components and provides information regarding the fundamental composition of a sample, inorganic and organic structure, biological molecules, quantitative and qualitative composition of composite structure, solid surface composition and atom isotopic ratio in sample (Skog et al., 2004[a]).

In many researches, Gas Chromatography-Mass Spectrometry (GC-MS) has been proven as an important scientific metabolic profiling in plant and non-plant species (De et al., 2013b; Kell et al., 2005; Sahaya Sathish et al., 2012).

GC-MS instruments separate chemical mixtures and identify the compounds at a molecular level. In GC a mixture would be separated into different substances once heated. The heated gases are passed within a column by an inactive gas like helium. The substances flow into the MS, when are separated from the column. Mass spectrometry detects compounds based on the molecule mass of the analyte as an electrical signal. That signal will be sent to a data system, which creates an image termed a chromatogram presenting the peaks of analyte (McNair & Miller, 2011).

A library of known mass spectra of several thousand substances is stored on a computer which is used to identify the unknown compounds (Harold et al., 2009).

2.5 Evaluation of Biological Activity

Medicinal plants are generally non-toxic than synthetics drugs due to their natural source and have been used traditionally as medicine to cure many diseases for long time. Isolation anticancer of natural products needs suitable bioassay to assist fractionation at every step. Several bioassay methods are invented for anticancer drug screening. A screening bioassay is functional to great number of original samples to detect those with preferred bioactivities. The need of utilizing the bioassay is to remove the passive constituents by fractionation of raw materials to isolate pure active substances.

Cytotoxic screening models offered significant initial data to choose extracts of plant with probable antineoplastic characteristics. The original screenings are cell-based assays utilizing proven cell lines, where the toxic impacts on extract of plants or isolated substances can be determined (Cardellina et al., 1999).

MTT assay is a based on color change of the cellular enzymes reacted on Tetrazolium dye, MTT, to produce purple color of insoluble Formazan. This assay is considered as one of the beneficial techniques has been used *in vitro* to measure the cytotoxicity of plant extracts. The MTT assay is inexpensive, less time requirement, non-complicated, typically reproducible, and does not need living things (Ames et al., 1995).

Colon cancer is one of the most dangerous types of cancer; its different stages warrant various treatment options such as chemotherapy, surgery, radiation and phytotherapy. Noteworthy, cancer therapeutics have adverse effects. For this reason, Plant compounds are advantageous for the treatment of colon cancer with additional benefit of improving (Kuppusamy et al., 2014; Prakash et al., 2012).

The incidence and mortality rates for colon cancer have been increasing in most of the countries, particularly US, European and part of Asian countries. This increasing incidence of colon cancer appears due to changing dietary constituents, physical activity patterns, as well as genetic influences (Center et al., 2009).

Most of the drug using for cancer treatment are found in numerous edible plants and vegetables such as green pepper, celery and perilla. Naturally isolated those drugs from secondary metabolites are clinically proven to be effective colon cancer agents. In vivo most of the studies showed that those drugs can decrease tumor incidence and multiplicity. (Lim et al., 2007; Yoshida et al., 2007).

Plant derivatives such as secondary metabolites have a major role in health and disease. These are reasonable way to maintain health and avert the formation of colon cancers.

Figure 2.2 shows clinically important secondary metabolites and their analogous structures.

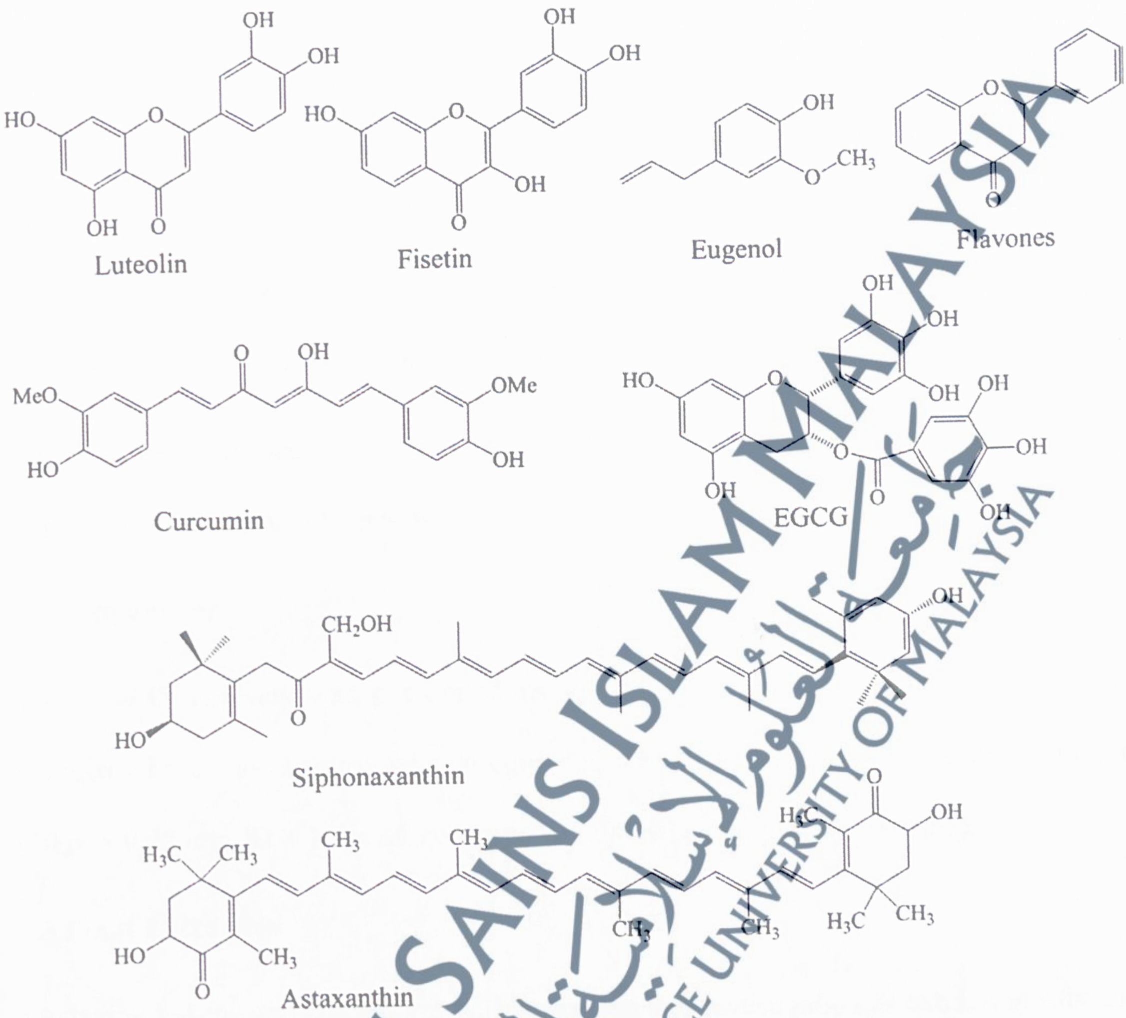

Figure 2.2: Structures of clinically important secondary metabolite used in colon cancer treatment (Kuppusamy et al., 2014).