GASTROINTESTINAL NEMATODES PARASITES OF GOATS (*Capra aegagrus hircus*) IN MERBOK, KEDAH, MALAYSIA: STUDIES OF ANTHELMINTIC EFFICACY OF SELECTED PLANTS, PREVALENCE AND NATURE OF INFECTION

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UNIVERSITI SAINS MALAYSIA

2018

GASTROINTESTINAL NEMATODES PARASITES OF GOATS (*Capra aegagrus hircus*) IN MERBOK, KEDAH, MALAYSIA: STUDIES OF ANTHELMINTIC EFFICACY OF SELECTED PLANTS, PREVALENCE AND NATURE OF INFECTION

by

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Thesis submitted in fulfillment of the requirement for the degree of Master of Science

August 2018

ACKNOWLEDGEMENT

I would like to show my greatest appreciation to my supervisor, Assoc. Prof. Dr. Zary Shariman Yahaya, co- supervisor, Assoc. Prof. Dr. Nik Fadzly and my field supervisor, Dr. Chandrawathani from Department of Veterinary Services for their tremendous encouragement, contribution of stimulating ideas and guidance. Without their supervision and constant help, this dissertation would not have been possible.

Special thanks to Dr. Nik Ahmad Irwan Izzauddin Nik Him for allowed me to use their laboratory equipment and support given. I would like to thank Dr. Hamdan Ahmad, Coordinator Vector Control Research Unit for his helps during the field works, opinions and insightful comments. Also special thanks to Universiti Sains Malaysia and the government for funding and scholarship given.

My appreciation also goes to wonderful labmates Rajiv, Azirah, Firdaus, Mariani and the undergraduate student, Tan Weng Hong for their excellent cooperation and knowledge sharing. Not forgetting, Sumayyah, Saffawati, Safiah, Atikah, Khadijah, Farah Alia, and the technical staffs, as well as Encik Hafiz (owner of Belau Sekawan Farm) and his assistant, Basri in helping me throughout these challenging years.

To my family and love one, thanks for the non-stop lovable spirits, financial support and prayers; were what sustained me thus far. Last but not least, I thank my fellow colleagues especially ‘Geng Adik Manis’ and lovely G09A team for the stimulating discussions and for all the warmness and happiness we had together.

Fatin Amirah binti Firuz, August 2018

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LIST OF ABBREVIATIONS

%	Percentage
˚C	Degree Celcius
Cm	Centimeter
G	Gram
Ml	Milliliter
Mm	Millimeter
Rpm	Rotation per minutes
BLAST	Basic Local Alignment Search Tool
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic
EPG	Egg Per Gram
FAMACHA	Faffa Malan's Chart
FECRT	Faecal Egg Counts Reduction Test
GC-MS	Gas chromatography and Mass-spectrum
HPLC	High-performance liquid chromatography
L_3	Third larvae stage
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PSM	Plant Secondary Metabolites

PARASIT NEMATOD GASTRO USUS KAMBING (*Capra aegagrus hircus*) DI MERBOK, KEDAH, MALAYSIA: KAJIAN EFIKASI ANTIHELMINTIK TUMBUHAN TERPILIH, PREVALANS DAN CIRI-CIRI INFEKSI

ABSTRAK

Kerintangan nematod gastrousus (GIN) terhadap ubat anti-cacing merupakan masalah yang berpontensi memberi kesan terhadap kos pengurusan industri ternakan secara global. Situasi ini mendorong para penyelidik menghasilkan ubat antihelmin berasaskan tumbuhan herba. Saringan awal metabolit sekunder dengan menggunakan gas kromatografi dan spectrum jisim (GC-MS) dan kromatografi cecair prestasi tinggi (HPLC) telah dijalankan ke atas ketiga-tiga ekstrak tumbuhan; *Azadirachta indica*, *Clinicanthus nutans*, dan *Polyathia longifolia*. Beberapa metabolit sekunder daripada jenis gula yang dipercayai mampu meningkatkan imunostimulan adalah d-mannose bagi daun mambu dan D-fructose,3-O-metil-, 3-O-Metil-d-glukosa serta glukosa bagi daun ashoka. D-Allose ditemukan dalam ketiga – tiga ekstrak tumbuhan. Bagi HPLC, beberapa unsur yang dikenalpasti adalah fruktosa, galaktosa, glukosa dan rhamnose. Disamping itu, analisa fitokimia turut dijalankan untuk menentukan kehadiran alkaloid, flavonoid, tanin, saponin, triterpena dan steroid. Komponen dan kandungan fitokimia bagi ketiga – tiga tumbuhan herba ini jelas menunjukkan ciri – ciri etnoveterinari bilamana ia menyebabkan larva di peringkat ketiga lumpuh dan mati dalam ujian “*in vitro*”; ujian perencatan migrasi larva dan ujian keupayaan pergerakan larva. Kajian “*in vivo*” telah dijalankan bermula Mei sehingga Julai 2016 untuk menilai keberkesanan di antara ekstrak tumbuhan yang dipilih dan ubat antihelmintik (albendazole dan ivermectin). FECRT, pengkulturan tinja, persampelan kawasan ragut, serta analisa darah; FAMACHA©, peratusan jumlah sel, smer darah tebal dan smer darah nipis telah dijalankan ke atas 24 ekor ternakan di sebuah ladang persendirian di Merbok, Kedah. Dua daripada lima kumpulan rawatan yang menunjukkan pengurangan kadar peratusan epg (FECRT) adalah ivermectin (77.78%) dan mambu (10.18%). Walaubagaimanapun, keputusan FEC menunjukkan bahawa ekstrak daun Mambu yang paling berkesan antara ketiga – tiga tumbuhan yang dikaji dengan nilai $P<0.05$ berbanding kumpulan kawalan, dan penyusutan skor FAMACHA yang menyebabkan peningkatan nilai purata PCV daripa 20% (pra – rawatan) kepada 24.33% (pasca – rawatan). Proses pengecaman larva peringkat ketiga dijalankan dengan menggunakan teknik morfologi dan molekular. Spesis nematod yang paling banyak dikenalpasti daripada pengkulturan tinja dan persampelan kawasan ragut adalah *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp., dan *Oesophagostomum* spp.. Dua spesies yang dikenalpasti melalui ujian molekular ialah *Trichostrongylus colubriformis* (JF276021.1) dan *Haemonchus contortus* (KU558757.1). Malangnya, *Cooperia* spp. dan *Oesophagostomum* spp. tidak dapat dikenalpasti kerana jumlah larva yang terlalu kecil. Sehubungan dengan itu, kajian yang lebih mendalam perlu dilakukan dalam usaha menghasilkan rawatan berasaskan herba yang sesuai dan efektif sebagai alternatif kepada ubat antihelmin.

GASTROINTESTINAL NEMATODES PARASITES OF GOATS (*Capra aegagrus hircus*) IN MERBOK, KEDAH, MALAYSIA: STUDIES OF ANTHELMINTIC EFFICACY OF SELECTED PLANTS, PREVALENCE AND NATURE OF INFECTIONS

ABSTRACT

Anthelmintic resistance of gastrointestinal nematodes (GIN) is a potentially costly problem that affected the livestock industries worldwide. The emergence of resistance urges the researchers to come out with an eco-friendly anthelmintic based on medicinal plants. Preliminary secondary metabolite screening by using gas chromatography and mass spectrum (GC-MS) and high – performance liquid chromatography (HPLC) were done for three plants crude extract; *Azadiracta indica*, *Clinicantus nutans* and *Polyalthia longifolia*. Few secondary metabolites focusing on sugar believed to boost the immunostimulant were d-mannose for neem and D-Frutose, 3-O-methyl-, 3-O-Methyl-d-glucose and glucose for ashoka. D-Allose was found in all three plant extracts. For HPLC few constituents identified were fructose, galactose, glucose and rhamnose. In addition, phytochemical analysis was conducted to investigate the presence of alkaloids, flavonoid, tannins, saponin, triterpenes and steroids. The phytochemical constituents of all three plants were shown to exhibit ethnoveterinary properties as it caused paralysis and death of third stage larvae in the larval migration inhibition assay and larval motility assay. An *in vivo* study was carried out from May to July 2016 to evaluate the efficacy of between selected plants aqueous extract and anthelmintic drugs (albendazole and ivermectin). The FECRT, faecal culture, pasture sampling, as well as blood analysis; FAMACHA©, percentage packed cell volume (% PCV), thin and thick blood smears were conducted on 24 free grazing experimental animals in a private farm in Merbok, Kedah. Only two out of five treatment groups shown reduction of epg in FECRT; ivermectin (77.78%), neem (10.18%). While, FEC results indicated that neem leaf was the most efficacious among three plants tested ($P<0.05$) compared to control group, thus decreasing FAMACHA scores that resulted in increased average of PCV values from 20% (pre-treatment) to 24.33% (post-treatment). The identification of third stage larvae was performed based on morphology and molecular techniques. The most abundance nematodes species identified from faecal culture and pasture sampling infecting the animals were *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp. and *Oesophagostomum* spp.. Two species were identified through molecular test were *Trichostrongylus colubriformis* (JF276021.1) and *Haemonchus contortus* (KU558757.1). Unfortunately, *Cooperia* spp. and *Oesophagostomum* spp. could not been identified due to small number of larvae present. Therefore, further studies are urgently needed, in order to come out with reliable, appropriate and effective principal of herbal remedies as an alternative to anthelmintic drugs resistance.

CHAPTER 1

INTRODUCTION

Kedah is a state, which covers a total area of 9000 km^2 situated in the northwestern part of Penisular Malaysia, bordering Thailand and consists of the mainland and Langkawi Island. Small-holder farmers rear the majority of the goats and sheep populations in Malaysia. The total population of goats in Kedah steadily increase to 50 095 heads in 2015 compared 49 353 heads in 2014 (Department of Veterinary Services, 2017). Unfortunately, the ruminants such as goats and sheep are susceptible to helminthiasis (Chandrawathani *et al.*, 2013b; Rozita *et al.*, 2014). Helminthiasis is considered as one of the major constraints hindering the slow pace of growth of livestock production in tropical countries causing millions dollar of detriment each year.

Poor husbandry system practice such as hygiene and poor nutrition is an important contributing factor to the high strongyle infestation among the livestock animals especially when they are exposed constantly to larval pick-up when grazing during wetter periods of the year (Chandrawahani *et al.*, 2013a). *Haemonchus contortus*, the blood sucking parasite which belongs to the family Trichostrongyloidea causes significant losses that may lead to blood loss, dehydration and anaemia (Soulby, 1982; Rozita *et al.*, 2015). Heavy infections can have detrimental effect on domesticated animals such as goats and sheep leading to fatality of the host. Combination of clinical conditions and syndrome cause by nematode infections known as parasitic gastroenteritis (PGE) which is characterized by dull, diarrhea, depression, inappetance, anaemic, and emaciation among animals (Soulsby, 1982; Cringoli *et al.*, 2004; Rozita *et al.*, 2014).

Over the past few decades, goat farming in Malaysia has been afflicted by helminthiasis due to the development of anthelmintic resistant (Chandrawathani *et al.*, 2013a). In Malaysia, anthelmintic resistance in goats and sheep was documented in the early 1990s, with demonstration of resistance to benzimidazoles (Rahman, 1993;1994a; Pandey and Sivaraj, 1994; Premaalatha *et al.*, 2014), levamisole (Dorny *et al.*, 1994; Premaalatha *et al.*, 2014) and the occurrence of multiple anthelmintic resistance was first reported by Sivaraj and co-worker in 1994 (Sivaraj *et al.*, 1994; Premaalatha *et al.*, 2014). Such frequent usage of chemical drug results of deemed ineffective towards target species (Chandrawathani *et al.*, 2015).

Besides the development of anthelmintic resistance, the toxicity problems, chemical residue (Kaemmerer and Butenkotter, 1973; Akhtar *et al.*, 2000) and the recognition of the antigenic complexity of parasites have slowed vaccine development (Akhtar *et al.*, 2000). These problems has awakened the interest of herbal remedies as an alternative source of bioactive compound that are biodegradable into nontoxic products and potentially suitable for use in control of parasites and anthelmintic drugs (Waller, 1997; Vieira *et al.*, 1999; Kamaraj and Rahuman 2011). Admittedly, the introduction of ethnoveterinary plants will provide economical options to enable the farmers to use the most appropriate helminth control to suit their management system.

The herbal preparations can act as one of the alternative ways used to prevent or reduce the severity of disease (Murugaiyah, 2006; Chandrawathani *et al.*, 2013a). Neem or Margosa tree is a famous in Indian Ayurvedic medicine. Isolated compound of *Azadirachta indica* have been divided into two group, isoprenoids (which includes diterpenoids and tritepenoids containing protomeliacins, limonoids, azaddirone and its derivatives, geddunin and its derivatives, vilasinin type of compund and C-secomeliacins such as nimbin, salanin and azadiractin) (Biswas *et al.,* 2002). Azadirachtin, one of its biologically active constituents has been proven to inhibit 68% of *Haemonchus contortus* eggs from hatching (Chandrawathani *et al.*, 2013b).

Other than neem, medicinal remedies of interest that attract the attention of researchers from both biology and chemical views are *Clinicanthus nutans* and *Polyalthia longifolia*. In Malaysia, *Clinicanthus nutans* (Burm f.) Lindau, belonging to the family of Acanthecaea (Arullappan *et al.*, 2014) is a small shrub commonly known as "Belalai Gajah" or Sabah snake grass. It has been reported to have antioxidant, antiviral, anti-inflammatory and anticancer (Chin *et al.*, 2014). Isolated compounds of the leaves and stem of Sabah snake grass containing C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin 7- *O*-β- glucopyranoside, orientin, isoorientin and sulfur containing glucosides (Teshima *et al.*, 1998; Pannangpetch *et al.*, 2007).

On the other hand, *Polyalthia longifolia* belongs to the family of Annonaceae is a small medium size tree known as Asoka by the local. Traditionally, it has been commonly used to treat helminthiasis, fever, diabetes as well as in cardiac problems (Dixit *et al.*, 2014). According to Sampath and Vasanthi (2013), isolated compounds from Asoka leaves such as alkaloids, flavonoids, tannin, glycosides and volatile oils possess antimicrobial activity. While, methanolic extracts of leaves were tested for their antibacterial and antifungal potential.

Hence, the objectives of this present study were:

1) To determine the efficacy between three plants (*Azadirachta indica*, *Clinicanthus nutas* and *Polyalthia longifolia*) leaf extract against gastro
2) intestinal nematodes in goats.
3) To determine the secondary metabolite compounds in *Azadirachta indica*, *Clinicanthus nutans* and *Polyalthia longifolia*.
4) To determine the efficacy of leaf extracts and anthelmintic drugs against gastrointestinal nematode parasites in terms of faecal and blood parameters as well as the reduction of parasites infection symptoms.

CHAPTER TWO

LITERATURE REVIEW

2.1 Livestock Industry in Malaysia

2.1.1 Background of livestock industry

Malaysia's livestock industry includes animal such as sheep, goats, deer, cows and others is an important component of agriculture sectors, which commonly breed in the rural area with lots of plant vegetation, fruit farm, oil palm and rubber plantations, as well as at abandoned large field of grass for grazing (Siti and Firuza, 2011). Production of sheep and goats are mainly managed by small holder farmers, usually in a small scale. According to the Department of Veterinary Services, Malaysia, the small ruminant industry is projected to grow bigger due to higher demand of halal livestock based product with a total population of goats and sheep 439 667 and 140 049 heads respectively in 2015 (Department of Veterinary Services, 2017).

Traditionally, goats' skin is used to produce musical instruments such as 'gendang', 'kompang' and 'wayang kulit'. Nowadays, it is commercially used in the fashion industry. The livestock production is projected to grow bigger as the result of increasing demands of its products. Meat demand is expected to increase from 1.4 million tonnes in 2010 to 1.8 million metric tonnes by 2020 with 2.4 % growth per year (Ministry of Agriculture and Agro-Based Industry Malaysia, 2011)

Goat meat is not only essential to the gross national income, but it is also an important source of milk and meat products to the nation. Compared to cow's milk, goats' milk consist smaller size of globular protein which is easier to be digested.

This industry plays important role in food production and provide gainful employment.

2.1.2 Farm management problems faced by small holder farmer

Parasitosis has been acknowledged as one of the crucial determinants to livestock industry that cause major health and production problems worldwide. The infection by helminth depresses production of milk, feeding intake and weight gain. The combination of environmental stress and helminthiasis can lead to death among the ruminants. According to Cheah *et al.* (1997), the humid tropical environment of Malaysia provides most favourable conditions for the development of pre-infective larval stages. Helminth infection occurred during grazing activity when the animals graze on contaminated pasture.

Intensive and indiscriminate use of anthelmintic, both broad and narrow spectrum types lead to resistant problem. Drugs resistance over the past decade also was documented in both government and private farms (Chandrawathani *et al.*, 2004; Khadijah *et al.*, 2006a, 2006b; Basripuzi *et al.*, 2012; Thongsahuan *et al.*, 2014). Endoparasitism diseases and drug resistance in small ruminants mainly sheep and goats are threaten the viability of the industry.

2.2 Gastrointestinal Nematodes in Goats

2.2.1 Taxonomy of Common Veterinary Gastro-Intestinal Nematodes

Nematodes or known as roundworms constitute to various phyla; the Nemathelminthes or Aschelminthes in the past which now generally belongs to the phylum of Nematoda (Sutherland and Scott, 2010). Table 2.1 below shows the taxonomy of common veterinary gastrointestinal nematodes of goats.

Table 2.1: Taxonomy of common veterinary gastrointestinal nematodes of goats

Order	Superfamily	Family	Genus	Species
Strongylida	Trichostrongyloidea	Trichostrongyloidae	*Haemonchus*	*H. contortus, H. placei*
			Cooperia	*C. curticei, C. onchophora*
			Trichostrongylus	*T. Axei, T. colubriformis*
			Nematodirus	*N. battus, N. spathinger*
			Ostertagia	*O. ostertagi, O. circumcincta*
	Strongyloidea	Strongyloidae	*Strongylus*	*S. vulgaris, S. equinus, S. edentates*
		Charbetidae	*Oesophagostomum*	*O. radaitum, O. dentatum, O. columbianum, O. venulosum*

Adapted from Taylor, Coop and Wall (2007).

2.2.2 Species of nematodes infecting goats

A wide range of parasites mainly *Haemonchus contortus*, *Trichostongylus* spp., *Cooperia* spp. and *Oesophagostomum* spp. are found in goats and sheep in Southeast Asia. According to Koski and Scott (2001), these nematodes causes chronic pervasive infections that contribute to wide spread morbidity and mortality worldwide

2.2.2(a) Biology of *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp. and *Oesophagostomum* spp.

Most gastrointestinal nematodes which infect both sheep and goats have the same basic life cycle throughout their life. Basically, the life cycle (Figure 2.1) is divided into two stages which are the free-living stage and the infective or parasitic stage. After copulating, the eggs will be passed out in the goat's feces. The free-living stage starts when the eggs hatch and continue growing from L_1 up to the non-feeding infective stage (L_3), which subsequently migrate onto the herbage and infects livestock during grazing activities. According to Gordon (1948), the third stage larvae are an active stage and migrate under the influences of moisture, temperature, light and possibly gravity.

The L_3 can be transmitted to definitive host either via oral route. The infective larva will undergo two further molts (L_4 and L_5), mature and develop into adult worms inside the host in 15-21 days for most common species. The site of infection differs between nematode species (Crofton, 1963). *Haemonchus contortus* will infect the abomasum, *Trichostrongylus* spp. infects the small intestine, *Cooperia* spp. attack the base of small intestine and the large intestine will be occupied by the *Oesophagostomum* spp.. The newly developed mature adult male and female undergo copulation and the cycle begins again (Hendrix, 2006).

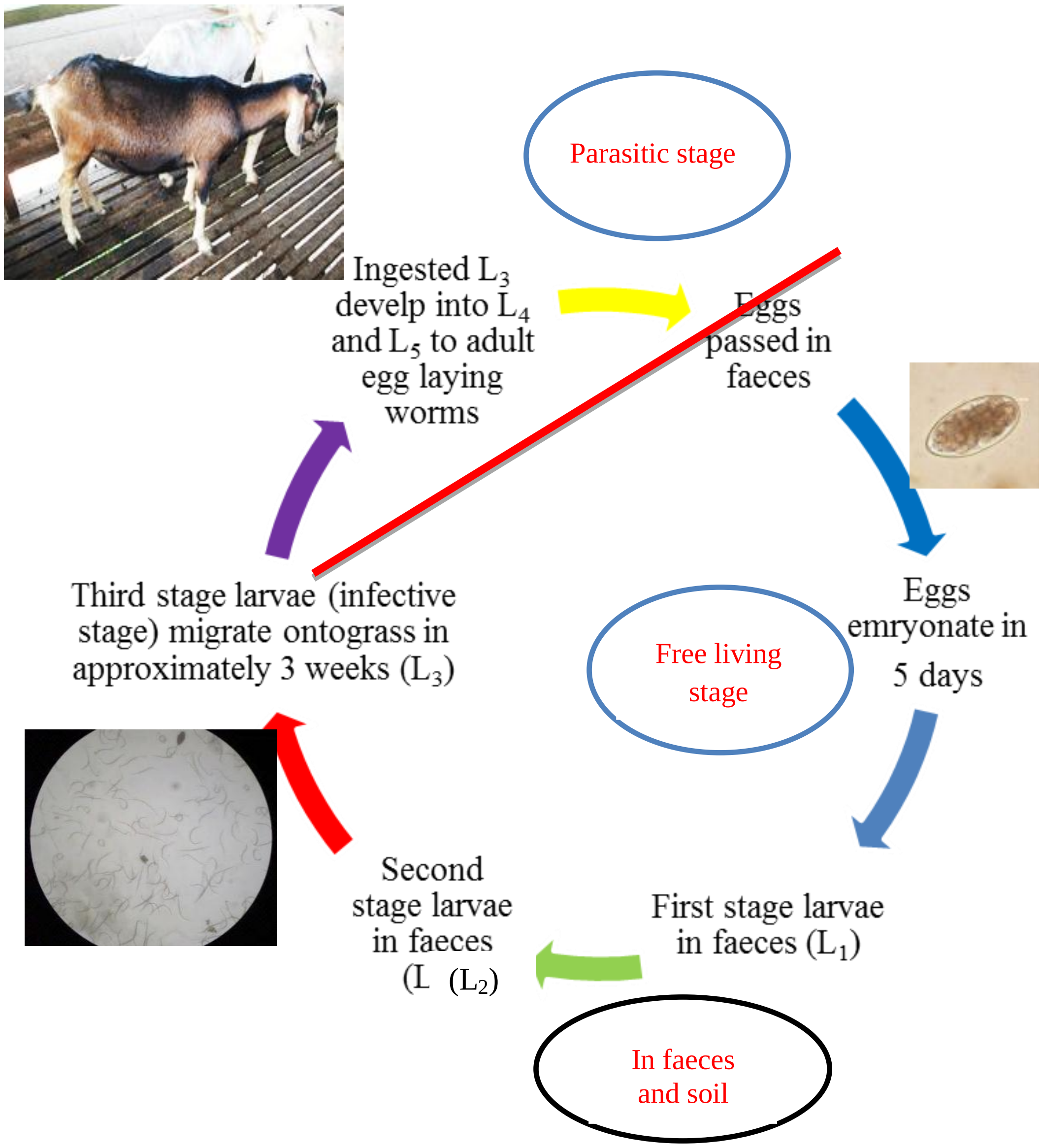

Figure 2.1: The general life cycle of trichostrongylid parasite.

2.2.2(b) Morphology of *H. contortus*, *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum* spp.

A. *Haemonchus contortus*

Haemonchus contortus is the most pathogenic gastro-intestinal parasites which can be found in the abomasum, the fourth stomach of a host system. Their blood-filled intestines spiral from anterior to posterior (tail) end is the reason why this species are called Barber's pole worm. The adult of Barber's pole worm is primarily a nematode of goats and sheep which feeds on blood and causes significant production losses due to morbidity and mortality (Rahman *et al.*, 2007). Other common names of *H. contortus* includes the "twisted stomach worm" and "wire worm" which characterized by the matured females' having white ovaries that wrapped around red, blood-filled intestine (Sutherland and Scott, 2010).

The larvae of this species has the most narrowly rounded head of the common larvae. The tail sheath is medium in length ending in a fine point and often has a slight kink at the end of the tail (Zajac and Conboy, 2012). The adult females are 20 – 30 mm long, had large "linguiform" or pendulous vulva flaps (Slocombe, 1974) and a prolific egg layers, deposited from 5 000 to 10 000 eggs per day. Whereas the males are much smaller compare to females around 15 mm long.

B. *Trichostrongylus* spp.

Contrary to *H. contortus*, the size of adult *Trichostrongylus* spp. is smaller; 4 – 6 mm long (males) and 5 – 7 mm long (females). Adult hair-worm can be identified by having prominent notch in the cuticle at the excretory pore. These scour worms parasitize small intestine of ruminants, pigs, horses, rodents, birds as well as humans (Schmidt, Roberts and John, 2009).

Morphologic characteristics of its infective third stage larvae are having overall length from 622 – 796 µm, rounded head, tail sheath is short and may have one or two rounded prominence known as tuberosities (Zajac and Conboy, 2012).

C. *Cooperia* spp.

Cooperia spp. is a common haematophagous parasitic nematode of goats, sheep, cattle and other wild and domestic ruminants worldwide. These small intestine roundworms are more abundant in regions with warm and humid weather. The most distinguishing morphological features of the genus are the cephalic vesicle and cuticle transverse striations, males present with short spicules with the absence of gubernaculum and a protuberant vulva in females (Amarante *et al.,* 2013).

The morphologic characteristics of third-stage larvae are 711 – 924 µm in length, squared head with two refractile oval bodies at anterior end of the esophagus and medium length of sheath tail tapering or finely pointed (Zajac and Conboy, 2012).

D. *Oesophagostomum* spp.

Oesophagostomum spp. also called “nodular worms” because of the appearance of characteristics nodules in the walls of both small and large intestine of their host form by the juveniles (Schmidt, Roberts and John, 2009). Adult of this species is 15 to 20 mm long. These nematodes can be found worldwide, but more frequent in tropical and subtropical region which are warm and humid climates.

Nodular worms third-stage larvae are larger than other species having the overall length 771 – 923 µm, rounded head, long thin sheath tail and 16 – 24 triangular intestinal cells (Zajac and Conboy, 2012). Common animals serve as the definitive host are goats, sheep, swine, cattle as well as the non-human primates, but human may also serve as definitive host. The third – stage larvae will burrow into the submucosa of the small or large intestines after ingestion. Digestion and defecation of host can be affected; diarrhea, enteritis and peristaltic movements are the clinical signs of oesophagostomiasis.

2.2.2(c) Ecology, epidemiology of ruminants' nematodes and their site of infection.

The climatic factors; humidity and temperature are the superior influences on the free-living stages of gastrointestinal nematodes such as *Haemonchus contortus* and *Trichostrongylus colubriformis*, with the effect of pasture conditions playing a significant modulating role (O'Connor, Walkden-Brown and Khan, 2006). Each parasite species that infect the host requires a different length of development period depending on the biotic factors. Temperature and humidity affect the dispersal and survival of L_3 on pasture.

To avoid desiccation, the third stage larval will migrate from faeces to pasture vegetation and accumulate in the soil or at the basis of vegetation during the day (Manfredi, 2006). Even though, the influences of temperature and moisture are less important once the infective stage is reached, the hot and dry conditions can be lethal to for these GIN infective larvae. The epidemiologically important gastrointestinal nematodes of goats, their site of infection and the consequences of parasitism are listed in Table 2.2 below.

Table 2.2: Epidemiologically important gastrointestinal parasites of goats, their site of infection and main effect on the parasitized host

Site of infection	Nematodes	Consequences of parasitism
Abomasum	*Haemonchus contortus*	Anaemia
	Teladorsagia circumcincta	Reduced food intake
Small intestine	*Nematodirus battus*	Dehydration
	Trichostrongylus colubriformis	Reduced food efficiency
	Trichostrongylus vitrinus	Reduced food efficiency

(Source: Athanasiadou *et al.*, 2009)

2.3 Anthelmintic resistance

2.3.1 Common anthelmintic used in farms

In livestock industry, anthelmintic is a well-known conventional chemotherapy method used to control most helminthes. Most of the helminthes have adapted with many strategies in their host and able to modulate or evade the immune response of the host for survival, which best described as the host-parasite relationship (Chandrawathani *et al.*, 2013a). There are variety of chemical drugs categorized as anthelmintics such as benzimidazoles, levamisole, avermectin and milbemycins, piperazine, morantel, closantel, morantel and emodepside (Holden-Dye and Walker, 2005). The anthelmintics are groups into four, which are levamisole, benzimidazole, macrocylic lactone and amino acetonitrile derivative (EAFRD, 2011). Benzimidazoles, levamisole and closantel are the most commonly used to control parasite infection in Malaysia (Chandrawathani *et al.*, 2013c). Thiabendazole was the first highly efficacious, wide-range anthelmintic produced (Brown *et al.*, 1961).

2.3.2 Mode of actions

Other drugs such as ivermectin, albendazole, and diethylcarbamazine (DEC) were known to kill the microfilarial stage of parasites and have low level of activity in adult worms (Weil *et al.*, 2008). Albendazole and mebendazole are two of the common drugs which effective against nematodes, while DEC was widely used in treating lymphathic filariasis, which lead to death of microfilariae or harm the adult worms (De Silva *et al.*, 1997). Two major classes of drugs are the broad spectrum commercially released namely benzimidazole / probenzimidazoles, tetrahydropyrimidines / imidazothiazoles, and macrocyclic lactones, while the narrow spectrum anthelmintics include organophosphates, substituted phenos, and salicylanilides.

The mode action of benzimidazoles is by attaching to tubulin dimers, preventing their polymerization to microtubules resulted in the disassembly of existing cytoplasmic microtubules structures of the parasites (Prichard *et al.*, 1978; Chandrawathani, 2004). These actions reduce the parasite's nutrient absorption. Tetrahydropyriidines / imidazothiazoles suc as morantel and levamisole cause rapid paralysis of parasite. On the other hand, macrocylic lactones are potent insecticides and nematocides. For example, ivermectin is a potent inhibitor of the motility and development of the free-living stages of trichostrongylids (Campbell, 1983; Chandrawatani, 2004). Closantel is one of the members of narrow spectrum drugs which is highly effective against the *H. contortus* parasites for a month after treatment and significantly remains after 6 days (Hall *et al.*, 1981; Chandrawathani, 2004), which the worm acquire the anthelmintic become tightly bound to plasma protein.

2.3.3 Gastrointestinal nematode parasite resistance towards anthelmintic

The widespread use of drug anthelmintic to combat the gastrointestinal nematodes in the small ruminants has resulted in the occurrence of anthelmintic resistant not only in Malaysia but globally. In the early 1990s, the emerging threat of various anthelmintic resistance among the small ruminants especially goats and sheep was reported, with studies of resistance to the benzimidazoles (Rahman, 1993, 1994a; Pandey and Sivaraj, 1994; Premaalatha *et al.*, 2014), levamisole (Dorny *et al.*, 1994; Premaalatha *et al.*, 2014), imidazothiazoles, macrocylic lactones, salicylanilides (Pandey and Sivaraj, 1994; Rahman, 1994a; Sivaraj *et al.*, 1994; Dorny *et al.*, 1994; Nor-Azlina, 2010; Basripuzi *et al.*, 2012) and the first case of multiple anthelmintic resistance was reported in 1994 (Sivaraj *et al.*, 1994). Dorny *et al.* (1994) stated the presence of benzimidazole resistance in 33 out of 96 goat farm and while, Rahman (1994a) and Chandrawathani *et al.* (1999) reported levamisole resistant nematode population in 10 goat farms in Penang.

According to Varady *et al.* (2011), the occurrence of multiresistant population of all types of broad-spectrum chemical drugs in goats has been reported. Multiple resistance were reported in one goverment sheep breeding farm towards levamisole, oxfendazole, and ivermectin with suspected resistance towards moxidectin (Chandrawatani *et al.*, 2013c). Most of the manufacturer companies may not state the limitations of the product against gastrointestinal parasites of goats, which largely seem to be due to the costs involved in registration and may cause side effects to the animals. Goats are generally more susceptible to the same nematode as those of sheep, but increasing the dose rate of anthelmintics, particularly levamisole, can provoke toxicity (Chandrawathani *et al.*, 2004). A weaker infected animal with deficiency should be treated differently compared to other animals.

2.4 Plants as eco-green method to control gastro-intestinal nematodes in livestock

Researches on various ethnoveterinary plants as alternatives to commercially available anthelmintic are rapidly increasing in order to combat resistance (Dorny *et al.*, 1994; Rahman 1994b; Pandey and Sivaraj, 1994; Sivaraj *et al.*, 1994; Chandrawathni *et al.*, 1994; Rahman 2016) and significantly reduced nematode parasite in small ruminants. Small-holder farmers in developing countries can grow their own herbal plants and produce simple and nontoxic anthelmintic for livestock. Three species of plants used in this current study are *Azadirachta indica*, *Clinicantus nutans* and *Polyalthia longifolia*.

2.4.1 *Azadirachta indica*

Azadirachta indica A. Juss. (Plate 2.1) belongs to Meliaceae (mahogany) family has been identified as one of the potential environmental friendly anthelmintic. It is a fast growing hardy plant which may reach a height of 25 meters (Schmutterer, 1990) and widely distributed in dry areas of tropics and subtropics (Siddiqui *et al*., 2006) with annual rainfall of 400 – 800 mm and extended dry season, even with poor soils (Schmutterer, 1990; Atawodi and Atawodi, 2009). It is also known by the local in Malaysia as “Pokok Mambu”, each part of it has some medicinal property and thus commercially exploitable (Biswas *et al.*, 2002).

Growing research attention attributed to this promising medicinal plants focusing on biological, pharmacological and anthelmintic activities of the neem compound in controlling the helminthiasis problem in small ruminants. Neem seed kernel consists of azadirachtin and other limonoid and is known to possess anti-feedant, attractant, repellant, growthdisrupting and larvicidal properties against large number of pests (Kaushik *et al.,* 2007). One of its well-known biologically active constituents; azadirachtin has been proven to inhibit 68% of *Haemonchus contortus* eggs from hatching (Chandrawathani *et al.,* 2013b).

Besides the identification of secondary metabolites (Figure 2.2) such as azadirachtin, there are more than 135 compounds have been isolated from different parts of neem which have been divided into two major classes; isoprenoids (includes diterpenoids and tritepenoids containing protmeliacins, limonoids, azaddirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin) and others (Biswas *et al.*, 2002).

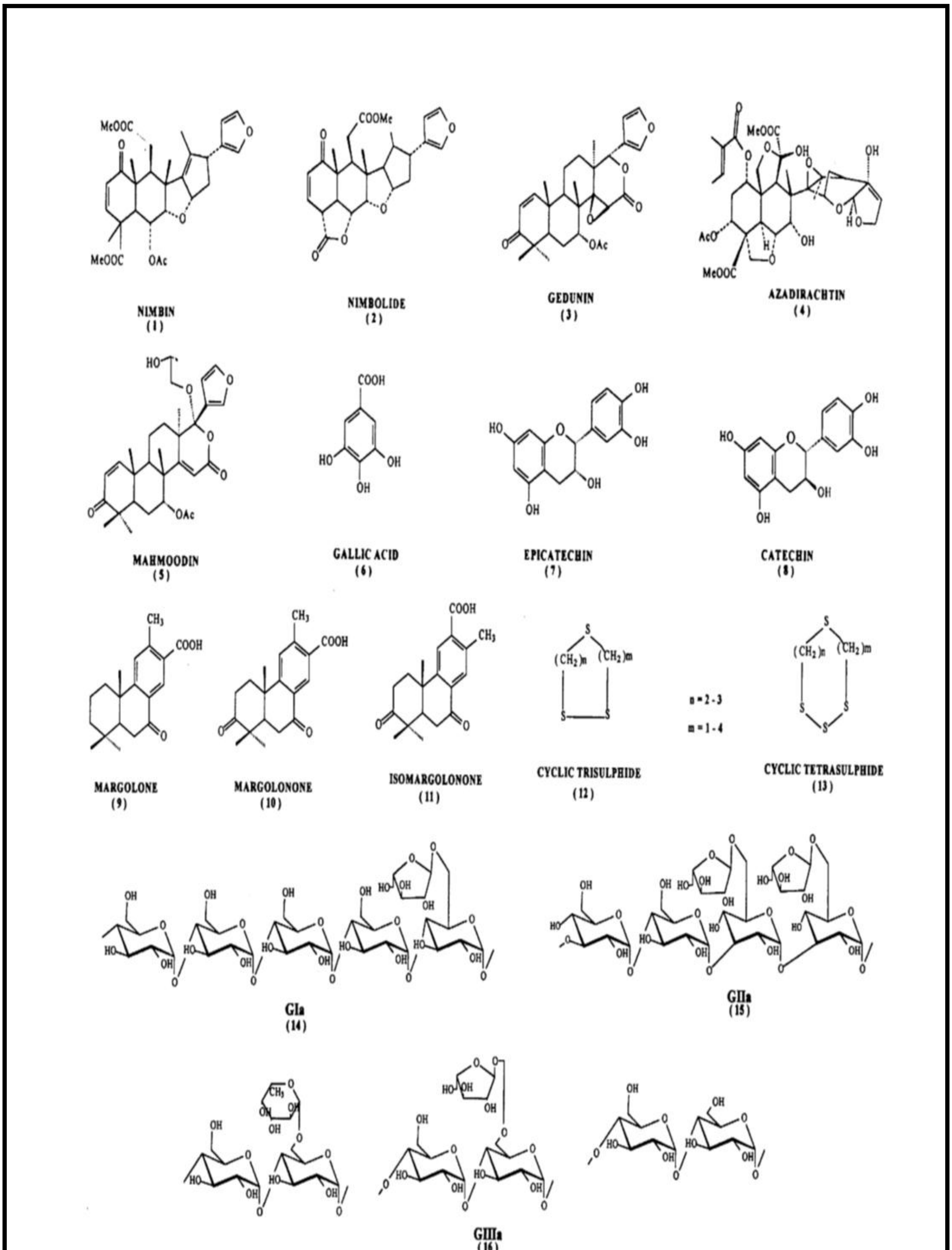

Figure 2.2: Isolated compound of *Azadirachta indica*

Adapted from Biswas *et al.* (2002)

Plate 2.1: *Azadirachta indica* trees along the USM roadside.

Plate 2.2:The leaves of *Clinacanthus nutans*

2.4.2 *Clinacanthus nutans*

Clinacanthus nutans (Burm. f.) Lindau is a small shrub belonging to the family Acanthaceae which is cultivated and growing wild in most tropical Asian countries. Locally known in Malaysia as belalai gajah or Sabah snake grass and Phaya Yo or Phaya Plong Thong in Thai (Sakdarat *et al.*, 2009; Kongkaew and Chaiyakunapruk, 2011), can grow up to 1 meter tall with pubescent branches, simple; narrowly elliptic-oblong or lanceolate (2.5 – 13.0 cm long x 0.5 – 1.5 cm wide) leaves (Plate 2.2) (Alam *et al.*, 2016).

This perennial herb is a diverse and potential medicinal uses as natural medicine in Malaysia, Indonesia and Thailand for treating skin rashes, insects, scorpion and snake bites, diabetes mellitus, fever and diuretics (Alam *et al.*, 2016). For many years, this plant has traditionally been used as Thai traditional herbal medicines for the treatment of herpes simplex virus (HSV) both type 1 and type 2 and varicella zoster virus (VZV) lesions (Sakdarat *et al.*, 2009; Vachirayonstien *et al.*, 2010; Kongkaew and Chaiyakunapruk, 2011; Alam *et al.*, 2016) diagnosed in patients. This species not only shown to inhibit herpes viruses, the pharmacological test of *C. nutans* proved to exhibit a broad range of biological properties such as antiviral, anti-diabetic, antioxidant, and anti-inflammatory activities (Alam *et al.*, 2016) as well as analgesic activity (Kongkaew and Chaiyakunapruk, 2011).

Previous studies indicated the presence of flavonoids such as belutin, vitexin, isovitexin, orientin, isoorientin, shaftoside, and isomollupentin-7-O-b-glucopyranoside (Wanikiat *et al.*, 2008; Khoo *et al.*, 2015; Alam *et al.*, 2016). According to Sakdarat *et al.* (2009), three pure compounds were isolated from *C. nutans* which were identified as chlorophyll a and chlorophyll b related compounds (Figure 2.3). The *n*-BuOH soluble fractions from methanolic stem and leaves extracts contain five glucosides (Teshima *et al.*, 1998; Alam *et al.*, 2016). Other compounds reported are sulfur-containing glucosides, glycoglycerolipids, pheophytins, sterols and cerebrosides (Teshima *et al.*, 1998; Sakdarat *et al.*, 2009; Khoo *et al.*, 2015). Figure 2.4 shows the structural of other bioactive compounds present in *C. nutans*.

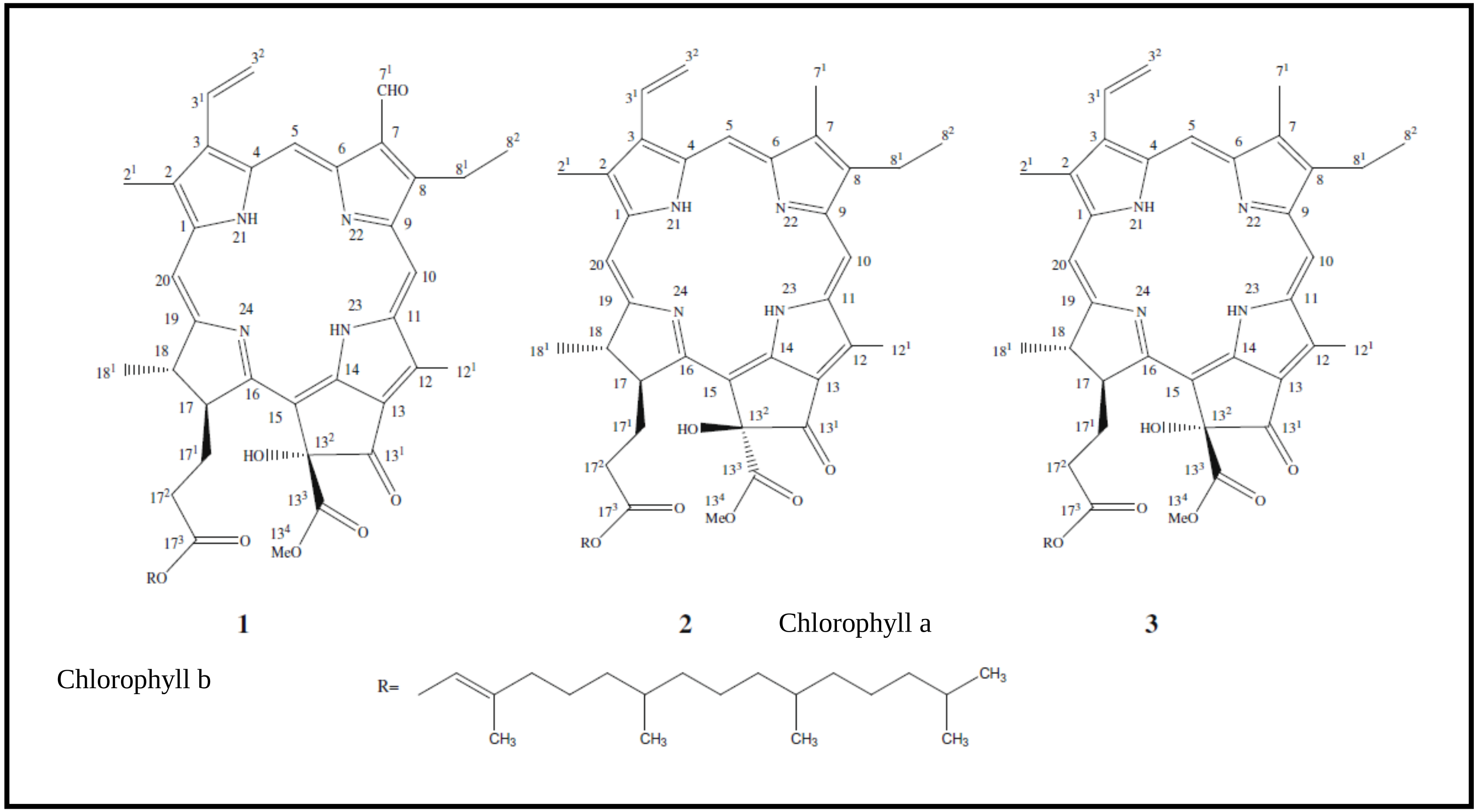

Figure 2.3: Structure of compounds 1-3 were identified as chlorophyll a and chlorophyll b related compounds (Sakdarat *et al.*, 2009).

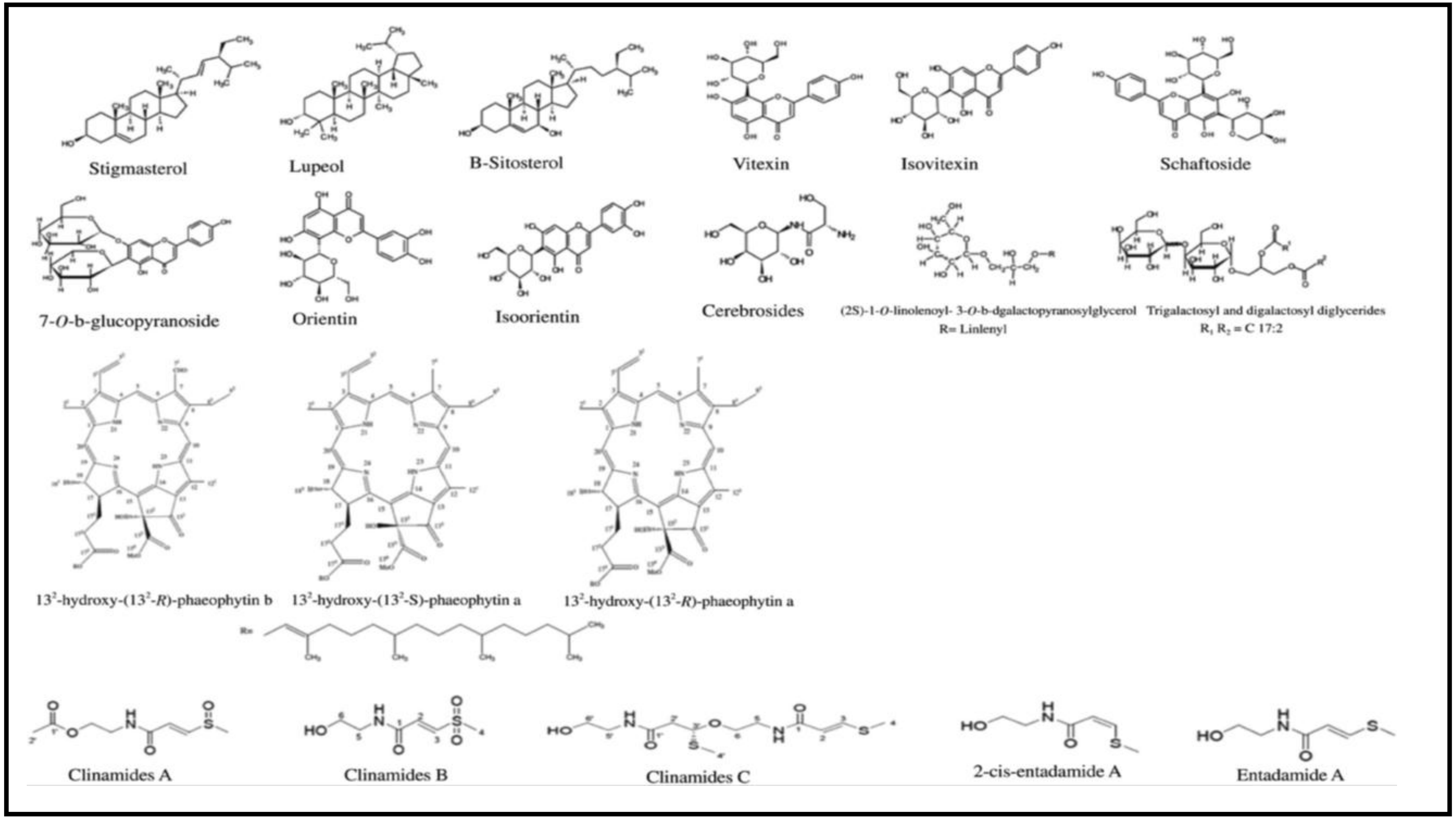

Figure 2.4: Structure of different bioactive compounds of *C. nutans* (Alam *et al*., 2016)

2.4.3 *Polyalthia longifolia*

Polyalthia longifolia (Sonn.) Thw. var. *agustifolia* (false Ashoka) from the family of Annonaceae is a tall evergreen tree (Plate 2.3), which cultivated in tropical part of India. It exhibits symmetrical pyramidal growth with willowy weeping pendulous branches (Dixit *et. al.*, 2014), 1 to 15 cm broad lanceolate leaves and known to grow over 30 feet in height. Other common names for *P. longifolia* are Green champa, India fire tree, Buddha tree, and Indian mast tree. *P. longifolia* leaves are aromatic and often used for decoration (Dixit *et. al.*, 2014).

This herbal plant has been used in medicoculturally worldwide for the treatment of helminthiasis, fever, diabetes, skin disease, and hypertension (Kirtikar and Basu, 1995; Chanda *et. al.*, 2012; Jothy *et. al.*, 2013a). It is also used as a tonic and febrifuge in traditional medicine (Jothy *et. al.*, 2013b). The bark is used as a folk lore medicine in treating pyrexia, diabetes, rheumatism, scorpion sting, menorrhagia (Jothy *et. al.*, 2013a) as well as other bleeding disorders (Dixit *et. al.*, 2014) including curing mouth ulcer in India. Extracts and isolated compounds from its stem bark were tested for various biological activities such as antibacterial, antifungal activity, and cytotoxicity (Jothy *et. al.*, 2013b).

A number of biologically active constituents (Figure 2.5) have been isolated from various parts of this versatile plant like alkaloids, flavonoids, diterpenes, sesquiterpenes, and saponins (Sharma *et. al.*, 2011). Its bark has been reported to contain steroid, diterpenes, alkaloids, and miscellaneous lactones (Jothy *et. al.*, 2013b). Clerodane and enihalimane diterpines from hexane extract of the stem bark of *P. longifolia* have demonstrated significant antiulcer, anti-inflammatory, antifungal and antibacterial activities (Sharma *et. al.*, 2011). Figure 2.5 shows the various phytochemical compounds isolated from *P. longifolia*.

Plate 2.3: *Polyalthia longifolia* tree along USM roadside.