ANTIPROLIFERATIVE PROPERTIES OF *CLITORIA TERNATEA* AND *VITEX NEGUNDO* ON CANCER CELL LINES

by

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LIST OF ABBREVIATIONS

ACS	American Cancer Society
ADA	American Dietetic Association
ANOVA	Analysis Of Variance
ATCC	American Type Culture Collection
As	Arsenic
B	Boron
Cd	Cadmium
Ca	Calcium
CNS	Central Nervous System
Cr	Chromium
CT	*Clitoria ternatea*
Co	Cobalt
Cu	Copper
DMEM	Dulbecco's Modified Eagle's Medium
BME	Eagle's Basal Medium
EDTA	Ethylenediaminetetraacetic Acid
FBS	Fetal Bovine Serum
GC-MS	Gas Chromatography–Mass Spectrometry
MCF-7	Hormone-dependent breast cancer cell line
Hela	Human cervical cancer cell line
Hs27	Human foreskin fibroblast cell line
HepG2	Human liver cancer cell line
Caov-3	Human ovary cancer cell line

HCl	Hydrochloric Acid
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrophotometer
INFORMM	Institute For Research In Molecular Medicine
Fe	Iron
Pb	Lead
Mg	Magnesium
Mn	Manganese
MTT	Micro-Culture Tetrazolium Salt
MEM	Minimum Essential Medium
NCI	National Cancer Institute
Ni	Nickel
NADH	Nicotinamide Adenine Dinucleotide
MDA-MB-231	Non-hormone-dependent breast cancer cell line
AOAC	Association of Official Analytical Chemists
PBS	Phosphate Buffer Solution
K	Potassium
Se	Selenium
Na	Sodium
NaOH	Sodium Hydroxide
SE	Standard Error
VN	*Vitex negundo*
WHO	World Health Organization
Zn	Zinc

DAN SIFAT –SIFAT ANTI-PROLIFERATIF *CLITORIA TERNATEA* DAN *VITEX NEGUNDO* KE ATAS SEL SELANJAR KANSER

ABSTRAK

Daun dan bunga *Clitoria ternatea* dan *Vitex negundo* dinilai dari segi analisis proksimat menggunakan kaedah AOAC. Kandungan mineral dan logam berat dalam kedua-dua tumbuhan ditentukan dengan menggunakan *Inductively Coupled Plasma Optical Emission Spectrophotometer* (ICP-OES). Tujuan utama kajian ini adalah untuk memeriksa aktiviti anti-proliferatif (nilai IC_{50} <100 μg/mL) ekstrak air dan metanol kedua-dua tumbuhan terhadap sel selanjar kanser, iaitu sel selanjar kanser payudara yang bergantung pada hormon (MCF-7), sel selanjar kanser payudara yang tak bergantung pada hormon (MDA-MB-231), sel selanjar kanser ovari (CAOV-3), sel selanjar kanser serviks (Hela), sel selanjar kanser hepar (HepG2)) dan sel selanjar bukan kanser, fibroblast kulit manusia (Hs27). Perencatan pertumbuhan sel diperiksa melalui asai MTT (3-(4,5-dimetilitiazol-2-il) 2,5- difeniltetrazolium bromida) untuk tempoh masa 24, 48 dan 72 jam. Hasil analisis proksimat mendapati daun dan bunga *Clitoria ternatea* dan *Vitex negundo* mengandungi fiber (2.1% ke 7%), karbohidrat (2.2% ke 18.9%), protein (0.32 ke 0.87%), lemak (2.5% ke 7%), abu (0.45% ke 8%) dan kandungan lembapan (53.4% ke 92.4%), masing-masing. Daun dan bunga *Vitex negundo* kaya dengan kalsium (10.5 dan 5.5 mg/g), masing-masing. Kesemua analisis logam berat (Ni, Cd, As, Pb) didapati berada dalam kepekatan rendah dan di bawah paras pengesanan, menunjukkan ia selamat untuk dimakan. Ekstrak metanol daun *Vitex negundo* didapati menunjukkan perencatan terkuat jika dibandingkan dengan ekstrak air terhadap sel selanjar kanser MDA-MB-

231 dengan nilai IC_{50} 65.38µg/mL ($P<0.05$). Analisis dengan kromatografi gas-spektrometri jisim (GC-MS) menunjukkan 20 puncak menandakan komponen metil utama (E)-fitol, β-ionone-methyl dan linoleik asid, yang dikenalpasti sebagai konstituen utama ekstrak tumbuhan yang bertanggungjawab terhadap aktiviti anti-kanser. Ekstrak air bunga *Clitoria ternatea* mempunyai kesan perencatan yang kuat jika dibandingkan dengan ekstrak metanol pada sel MCF7 dengan nilai IC_{50} 175.35µg/mL ($P<0.05$). Kromatografi GC-MS menunjukkan 5 puncak menandakan komponen utama iaitu mome inositol yang bertanggungjawab terhadap aktiviti anti-kanser ekstrak ini. Kajian ini jelas menunjukkan potensi ekstrak-ekstrak ini sebagai sumber bahan bioaktif yang boleh digunakan dalam industri makanan dan nutraseutikal. Selain itu, komposisi makanan kedua-dua tumbuhan mencadangkan potensi yang boleh digunakan dalam formulasi dan produk makanan yang baharu.

ANTIPROLIFERATIVE PROPERTIES OF *CLITORIA TERNATEA* AND *VITEX NEGUNDO* ON CANCER CELL LINES

ABSTRACT

Leaves and flowers of *Clitoria ternatea* and *Vitex negundo* were evaluated by proximate analysis using the AOAC method. The mineral and heavy metal contents in both plants were determined using the Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES). The major aim of this study was to examine the anti-proliferative activities (IC_{50} value <100 μg/mL) of water and methanol extracts of both plants against human cancer origin cell lines, namely MCF-7 (hormone-dependent breast cancer cell line), MDA-MB-231 (non-hormone-dependent breast cancer cell line), Caov-3 (human ovary cancer cell line), Hela (human cervical cancer cell line), HepG2 (human liver cancer cell line) and Hs27 (human non-cancer-origin fibroblast cell line). The cell growth inhibition was evaluated by the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay for 24, 48 and 72 hours. Results from the proximate analysis indicated that the leaves and flowers of *Vitex negundo* and *Clitoria ternatea* were composed of fiber (2.1% to 11.6%), carbohydrate (2.2% to 18.9%), protein (0.32 to 0.87%), fat (2.5% to 7%), ash (0.45% to 8%) and moisture (53.4% to 92.4%), respectively. Leaves and flowers of *Vitex negundo* were rich in calcium (10.5 and 5.5 mg/g), respectively. All the analysis of heavy metals (Ni, Cd, As, Pb) were found to be at a lower concentration and were below the detection limits, thus making the plants safer for consumption. The methanol extract of the leaf of *Vitex negundo* was found to have the strongest inhibitory effect in comparison with water extract on MDA-MB-

231 cancer cell lines with an IC_{50} value of 65.38µg/mL ($P < 0.05$). The gas chromatography-mass spectrometry (GC-MS) of this extract showed 20 peaks indicating that the major compounds were (E)-phytol, β-ionone-methyl and linolenic acid, which are recognized as constituents of plant extracts responsible for anti-cancer activity. Water extract of *Clitoria ternatea* flower had stronger inhibitory effect in comparison with methanol extract on MCF7 with an IC_{50} value of 175.35µg/mL ($P < 0.05$). The GC-MS chromatogram of this extract showed 5 peaks indicating that the major compounds was mome inositol, which might be most responsible for the anti-cancer activities of this extract. This study clearly indicated the potential of these extracts as the sources of bioactive compounds to be used in the food and nutraceutical industries. Moreover, the nutrition composition of both plant suggests the potential of using in new food products and formulations.

CHAPTER 1

INTRODUCTION

1.1 Research Background

In year 2007, the American Cancer Society (ACS) published that the total number of cancer mortality was around 7 million. The portions of this numeral represented by developing and developed countries were 38 and 62 percent, respectively (Garcia *et al.,* 2007). The World Health Organization (WHO) also has declared that total cancer deaths will rise from 7.6 million in 2005 to be 12 million in 2030 (Farooqui *et al.,* 2011). Statistics reported by American Cancer Society (ACS) about global cancer showed that death caused by cancer and new cases are likely to reach 17.5 and 27 million, respectively by year 2050 (Pan *et al.* 2010).

In developed countries, the ratio of breast, colorectal and uterus cancers were higher than those of the developing countries (Figure 1.1). In contrast, cervix, ovary, liver and the others had higher rates in developing countries (Othman, 2003). Nine most common types of cancer in women in the world in year 2000 are shown in Figure 1.1.

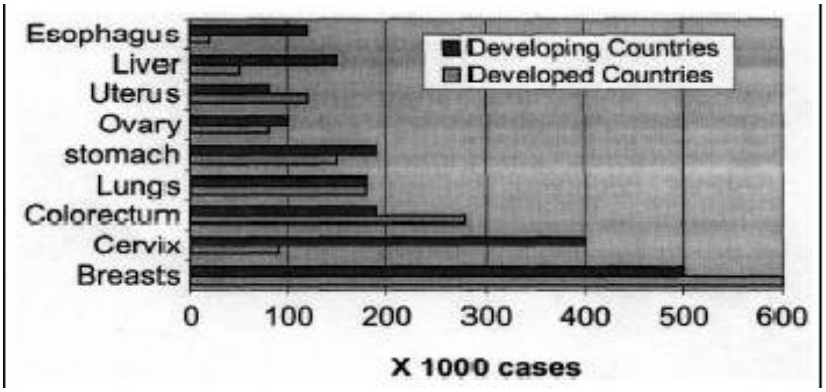


Figure 1.1 Nine common cancers affecting women in the world in year 2000 (Othman, 2003).

In year 2002, 26,089 of residing people in Malaysia were diagnosed for cancer including 14,274 female and 11,815 male (Musa *et al.,* 2011). Totally the chance of getting cancer for Malaysian was 1 in 4 persons for registered and unregistered cases (Lim *et al.,* 2003).

In 2006, the census population of Peninsular Malaysia was 24.8 million, of which 50.6% were male and 49.4% were female (Lim *et al.,* 2003). In year 2006 there were 21,773 cancer cases diagnosed in Peninsular Malaysia and registered at National Cancer Registry (NCR) (Musa *et al.,* 2011). These varied from breast, colorectal, lung, cervix uteri, liver and others. Figure 1.2 shows a clear picture of the ten most reported cancers in both genders in Peninsular Malaysia in year 2006. The breast, colorectal, lung, cervix uteri and liver had 16.5%, 13.2%, 9.4%, 4.9% and 3.6%, respectively of the total number, while other cancers were less than 3%.

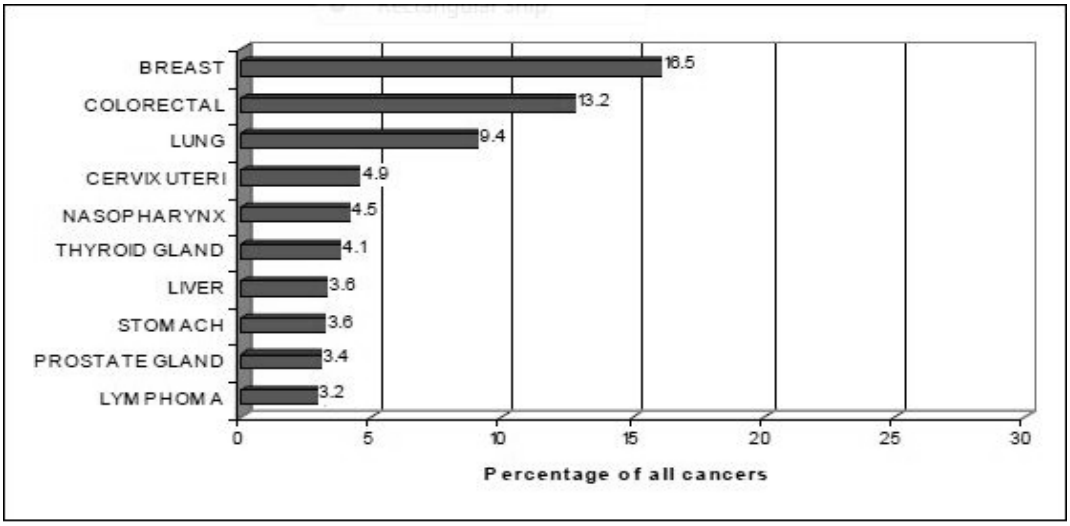


Figure 1.2 Ten most frequent cancers in both sexes, Peninsular Malaysia 2006 (Zainal, *et al.*, 2006).

The ten most common cancers among females in Peninsular Malaysia in year 2006 are shown in Figure 1.3. Percentage representations for breast, colorectal, cervix uteri and ovary cancer were 29.9%, 10.6%, 9.1% and 5.8%, respectively (Zainal *et al.*, 2006).

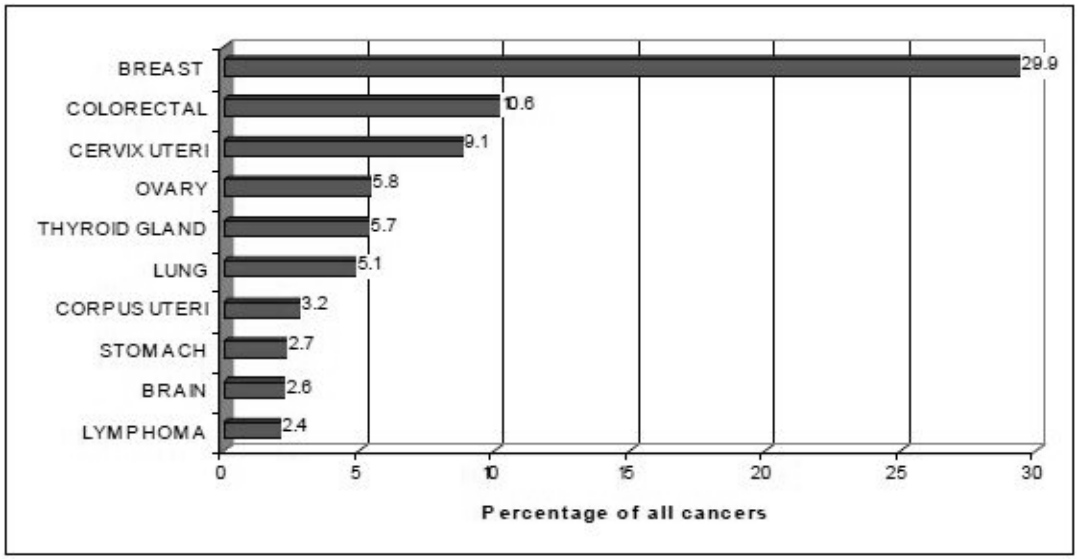


Figure 1.3 Ten most frequent cancers among females in Peninsular Malaysia 2006 (Zainal, *et al.*, 2006).

Lifestyle and nutrition related issues like obesity, smoking habit and dietary differences are associated with increasing the risk of developing cancer (Miller *et al.*, 1981). In case of family history of breast cancer many studies had shown that the risk of genetic factor is higher possibilities than other cancer (Musa *et al.*, 2011).

Currently, numerous methods for treating cancer exist such as chemotherapy, radiation, therapy, hormone therapy, immunotherapy and surgery. Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract, and hair follicles (Cooper, 1993). This results in the most common side-effects of chemotherapy are decreased production of blood cells, hence also immunosuppression, inflammation of the lining of the digestive tract and hair loss(Miller *et al.*, 1981). Some plants have ability to defeating cancers with fewer side effects compared to the other treatment (Wan-Nor Izzah *et al.*, 2009).

In this contemporary era in which we are experiencing radical changes in technology and medicine, cancers which still account for a large number of deaths annually in both developed and developing countries, remains a controversial issue between doctors and scientists (Pan *et al.*, 2010). There are considerable scientific and commercial interests in continuing the discovery of new anti cancer agents from natural product sources (Fouche *et al.*, 2008).

In 1989 Stephaen Defeliece defines the foods with some health benefits and prevention factor for different kind of diseases (Kalra, 2003). Hippocrates also noted

this many years ago that food can be effective for treatment of a variety of diseases and he mentioned that "*Let food be thy medicine and medicine be thy food*" (Bernal *et al.,* 2010). Nowadays, it has been believed that health status of an individual is really related to his/her good dietary routine. American Dietetic Association (ADA) in 2004 emphasized all kind of the food in particular dietary provides good health (Kant, 2004).

Based on the principal changes happened in human-health together with considering new approaches in the area of nutrition, the advantage of dietary intake has been more emphasized (Biesalski *et al.,* 2009). Due to certain practical limitations, the biological aspects and structural variegation of small molecule-secondary metabolites originated from nature are still not clear (Gesine *et al.,* 2010; Pan *et al.,* 2010). Studies toward exploring the powerful ability of biodiversity to improve health situation are currently increasing (Gesine *et al.,* 2010).

Clitoria ternatea (CT) is originally related to Fabaceae family. Worldwide it is known as butterfly pea and commonly known as Bunga telang in Malaysia. Flowers of *Clitoria ternatea* are used to make Nasi Kerabu which is a famous local dish in Kelantan. All parts of *Clitoria ternatea* are useful for medical treatments and have been used in folk medicines and for curing different diseases (Mukherjee *et al.,* 2008).

Vitex negundo (VN) is a member of Verbenaceae family which is also known as nirgundi, found mostly in India and Lemuni in Malaysia. Leave of this

plant is commonly used for making ‘Nasi Lemuni’ in the northern part of Peninsular Malaysia.

1.2 Rationale of Study

Over the years, medicinal plants species have been traditionally used to improve the physical well being of man and to treat several diseases worldwide. The health benefits provided by these traditional plants are attributed to the presence of phytochemicals with bioactivities. Nowadays, there is an increasing demand for treating diseases with healthy food supplements instead of using drugs. This is applicable to cancer treatment as well; especially as a variety of bad effects associated with cancer chemotherapy such as hair loss and weight loss. As such, scientists are trying to find the new methods to reduce the negative effects of these therapies on human bodies and develop the potential of natural treatments as anticancer (Wan-Nor Izzah *et al.,* 2009). However, in spite of the numerous benefits of the flowers and leaves of *Vitex negundo* and *Clitoria ternatea* against cancer cells, the available data about them is quite limited in Malaysia (Vishwanathan & Basavaraju, 2010). There have been no study in Malaysia objectively examine the antiprolifrative of crude extracts in the food such as flowers and leaves of *Vitex negundo* and *Clitoria ternatea*. This finding could be a good indicator for further *in vivo* study of these samples if the crude extracts show the lower IC_{50} value less than 100 µg/mL against specific cancer cell lines.

1.3 Research Objectives

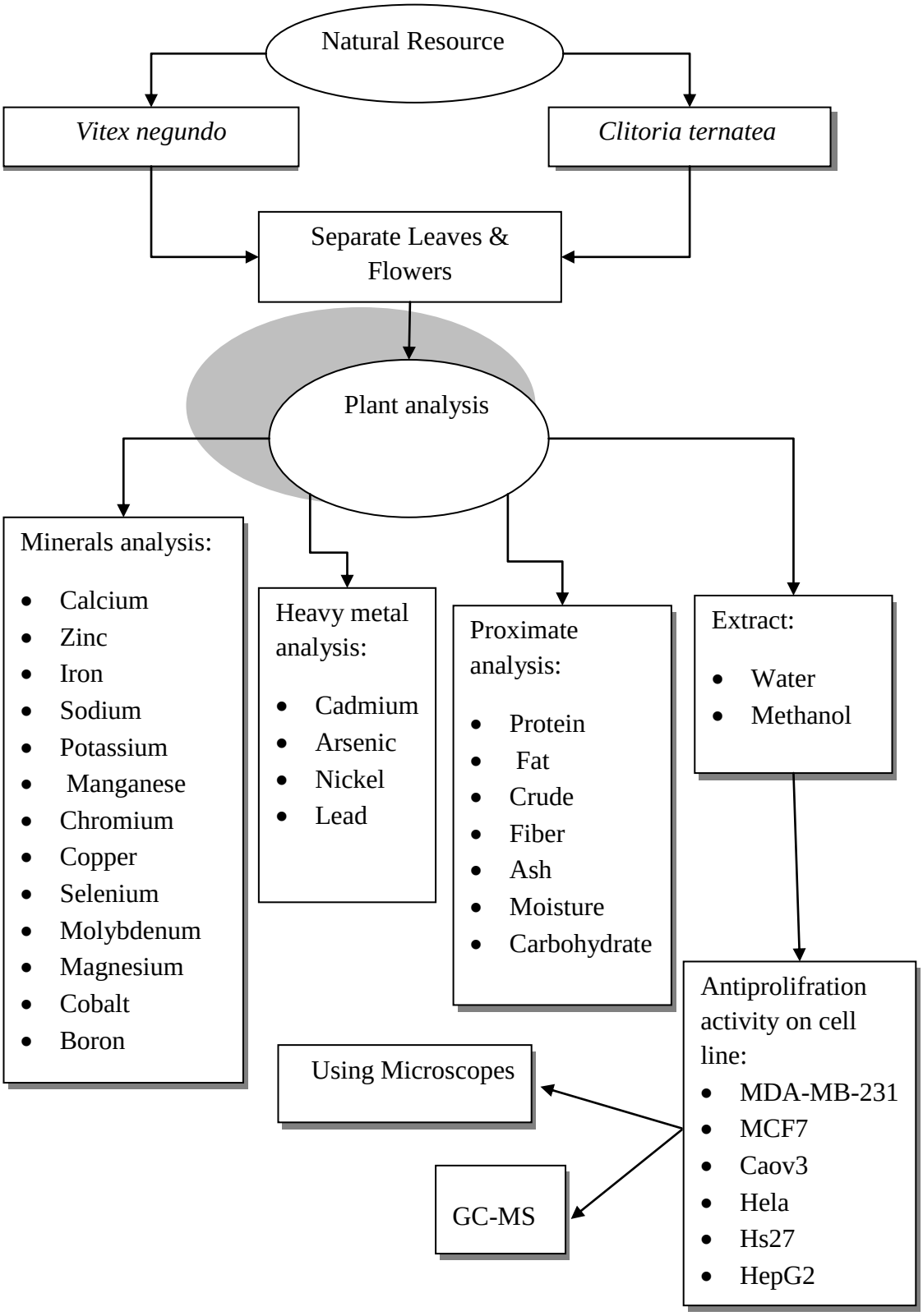
The main objective of this study is to evaluate the anti-proliferative properties exerted by the extracts of *Vitex negundo* and *Clitoria ternatea*

In specific terms, the objectives are:

- to determine the proximate compositions of *Vitex negundo* and *Clitoria ternatea* by using Association of Official Analytical Chemists (AOAC) Method and measure minerals and heavy metals contents of both plants using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES).

- to analyse the anti-proliferative properties of aqueous and methanolic extracts from these plants against human cancer cell lines using MTT (3-(4,5-dimetiltiazol-2-il)2,5 diphenyltetrazolium bromide) assay.

Conceptual framework



CHAPTER 2

Literature review

2.1 Medicinal Plant

The term "traditional medicine" mainly refers to Indian Ayurveda, Arabic Unani medicine and traditional Chinese medicine (Patwardhan *et al.*, 2005). The use of traditional medicine is still widespread and common among many Asian and South American communities based on historical circumstances and cultural beliefs (Vishwanathan & Basavaraju, 2010). About 40% of all health care delivery in China is via traditional medicine, while up to 80% of the population in Africa rely on traditional medicine for meeting their health care needs (World Health Organization, 2002; Vishwanathan & Basavaraju, 2010).

Folk medicines of almost all civilizations of the world are reliant on herbal remedies. Most of the traditional medicines utilized for the purpose of the healthcare are derived from plants (Kala *et al.*, 2004). Detailed clinical and pharmaceutical assessments have raised the status of medicinal plants due to the identification of the active components and principles present in them and by showing their mode of action in human and animal systems (Dutta, 1973). In general, benefit of using traditional plant includes less side effects, availability and affordability (Sharma *et al.*, 2008). However, Prajapti *et al.* (2003) emphasized a cautionary and important reminder that plant remedies are effective and without side-effects provided, they are selected properly and taken under proper medical supervision. It should be noted that, the active component, most often a secondary metabolite, varies in quality and

quantity for a given plant species growing in different locations. According to Purohit and Vyas (2004), close to 70,000 species of the plant kingdom have been used as herbal medicines.

2.2 Botany of *Vitex negundo* (VN) Species

Vitex negundo (VN) known also as Negundi throughout India and Lemuni in Malaysia, with gregarious growth in wastelands.This slender tree that stands erect with a height that usually ranges between 2–5m possesses quadrangular branchlets and is widespread in occurrence throughout India (Chopra *et al.,* 1956). The leaves are made up of five leaflets in a palmately arrangement, are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends. The plant has several bluish purple flowers and the fruit is soft, rounded and black in colour when ripe (Figure 2.1) (Tiwari & Tripathi, 2007).

Figure 2. 1 *Vitex negundo* plant

The plant is easily recognized by the purplish bottom part of the leaf (Chopra, *et al.,* 1956). The flowering period is from September to October and the flowers

produced are scented and possess both male and female organs (hermaphroditic) (Rastogi *et al.*, 2010). The plant's leaves are consumed as the principal component of the dish; Nasi lemuni (Figure 2.2), a popular local dish within the northern part of Peninsula Malaysia, while table 2.1 shows a taxonomical classification of *Vitex negundo*.

Figure 2. 2 Nasi Lemuni

Table 2.1 Taxonomical classification of *Vitex negundo* (Rastogi *et al.*, 2010)

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Verbenaceae
Genus	*Vitex*
Species	*Negundo*

2.2.1 Phytochemicals constituents

Higher plants store a wide range of bioactive constituents or phytochemicals that are useful in the pharmaceutical industry. According to Namdeo *et al.* (2007), a substantial proportion of all prescribed pharmaceuticals in developed countries are compounds which were directly or indirectly, derived from plants. The occurrence of phytochemicals or secondary metabolites usually in complex mixtures is usually different with regard to plant organs and stages of development (Banerji *et al.*, 1969; Wink, 2004). Adequate insight and information on phytochemical constituents are critical for evaluating the actual effectiveness of plants in medicine. Table 2.2 shows the details of the different phytochemical constituents that have been reported from different parts of *Vitex negundo* (Vishwanathan & Basavaraju, 2010).

Table 2.2 phytochemicals constituents in different part of *Vitex negundo* (Vishwanathan & Basavaraju, 2010)

Plant Part	Phytochemical Constituents
Leaves	hydroxy-3,6,7,3′,4′-pentamethoxyflavone 6′-p-hydroxybenzoyl mussaenosidic acid; 2′-p-hydroxybenzoyl mussaenosidic acid 5, 3′-dihydroxy-7,8,4′-trimethoxyflavanone; 5,3′-dihydroxy-6,7,4′-trimethoxyflavanone viridiflorol; β-caryophyllene; sabinene; 4-terpineol; gamma-terpinene; caryophyllene oxide; 1-oceten-3-ol; globulol betulinic acid [3β-hydroxylup-20-(29)-en-28-oic acid]; ursolic acid [2β -hydroxyurs-12-en-28-oic acid]; n-hentriacontanol; β-sitosterol; p-hydroxybenzoic acid protocatechuic acid; oleanolic acid; flavonoids angusid; casticin; vitamin-C; nishindine; gluco-nonitol; p-hydroxybenzoic acid; sitosterol
Seeds	3β -acetoxyolean-12-en-27-oic acid; 2α, 3α-dihydroxyoleana-5,12-dien-28-oic acid; 2β,3α diacetoxyoleana-5,12-dien-28-oic acid; 2α, 3β-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid vitedoin-A; vitedoin-B; a phenylnaphthalene-type lignan alkaloid, vitedoamine-A; five other lignan derivatives

Table 2.2 Continued

Plant Part	Phytochemical Constituents
	-6hydroxy-4-(4-hydroxy-3- methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3, 4-dihydro-2-naphthaldehyde β-sitosterol; p-hydroxybenzoic acid; 5-oxyisophthalic acid; n-tritriacontane, n-hentriacontane; n-pentatriacontane; n-nonacosane
Root	2β, 3α-diacetoxyoleana-5,12-dien-28-oic acid; 2α,3α-dihydroxyoleana-5,12-dien-28-oic acid; 2α,3β -diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid; vitexin and isovitexin negundin-A; negundin-B; (+)-diasyringaresinol; (+)-lyoniresinol; vitrofolal-E and vitrofolal-F acetyl oleanolic acid; sitosterol; 3-formyl-4.5-dimethyl-8- oxo-5H-6,7-dihydronaphtho (2,3-b)furan
Essential oil of fresh leaves, flowers and dried fruits	δ-guaiene; guaia-3,7-dienecaryophyllene epoxide; ethyl-hexadecenoate; α-selinene; germacren-4-ol; caryophyllene epoxide; (E)-nerolidol; β-selinene; α-cedrene; germacrene D; hexadecanoic acid; p-cymene and valencene.

Plant leaves contain an alkaloid nishindine, flavonoids like flavones, luteolin-7- glucoside, casticin, iridoid glycosides, an essential oil and other constituents like vitamin C, carotene, gluco-nonital, B-sitosterol, benzoic acid and C-glycoside (Akthar *et al.,* 1992). While hydrocarbons, B-sitosterol, benzoic acid and phthalic acid (Akthar *et al.,* 1992), anti-inflammatory diterpene, flavonoids, artemetin and triterpenoids can be found in the seeds (Chawla *et al.,* 1991; Chawla *et al.,* 1992). Fatty acids, B-sitosterol, vanilic acid, p-hydroxybenzoic acid and luteolin have been isolated from the bark (Akthar *et al.,* 1992). The stem bark also yields leucoanthocyanidins (Akthar *et al.,* 1992; Chopra *et al.,* 1956; Tandon, 2005). The various applications of *Vitex negundo* in traditional medicine have been grouped regionally to emphasize the ethnobotanical diversity and widespread occurrence of the plant; and the details are presented in Table 2.3.

Table 2.3 The Ethanobotanical diversity and widespread occurrence of *Vitex negundo* use in treatment of traditional (Vishwanathan & Basavaraju, 2010)

Country	State	Region	Local name	Use in treatment of traditional
India	Andhra Pradesh	Puttaparthi	Tella Vaavili	Asthma, Cancer Used as bath for women in puerperal state
India	Assam	-	Pochatia	Jaundice Urticaria, Cellulitis, Abcesses, Carbuncles, Eczema Liver disorders
India	Himachal Pradesh	Garwahl	Sambhaalu	Kwashiorkor
		Parvati valley	Bana	Wounds, Body ache
India	Karnataka	Dharwad	Lakki, Karilakki	Toothache
		Mysore	Bilenekki	Febrile, catarrhal and rheumatic afflictions
		Uttara Kanada	Nekki	Migraine
India	Maharashtra	Konkan	Lingur	Rheumatism
		Amravati	Samhalu	Encephalitis
		Chota Nagpur	Nirgundi	Expectorant
		Satpuda	-	Joint pain
India	Orissa	Malkangiri	Languni	Jaundice
India	Tamil Nadu	Southern parts	Notchi	Used as antidote for snake bite
		Madurai	-	Respiratory disorders, Fever, Headache
		Kancheepuram	-	Respiratory disorders, Fever, Headache
		Salem and Tirucchirappalli	-	Respiratory disorders, Fever, Headache
India	Uttar Pradesh	Jaunsar-Bawar hills	Somi	Eye pain
		Moradabad	Mala	Used as refrigerant for cattle
		Uttaranchal	-	48 types of ailments

Table 2.3 Continued

Bangladesh	Chittagong	-	Weakness, Headache, Vomiting, Malaria, Black fever
China	Guangdong	Buging′iab	Common cold, Flu and Cough
Nepal	Kali Gandaki	Simali	Sinusitis, Whooping cough
Pakistan	Buner	Marvandaey	Chest-pain, Backache,Used as toothbrush
	Kot Manzaray Baba valley	-	Used as anti-allergenic agent
	Margallah hills	Nirgud	Gum and skin diseases
	Siran valley	Kalgari	Used as medicine for buffaloes in colic
Philippines			Cancer
Sri Lanka	Nilnikka	Eye disease, Toothache, Rheumatism	Used as a tonic, carminative and vermifuge
Malaysia	Peninsular	Lemuni	Use to treat of rheumatism and postnatal woman

2.2.2 Bioactive compounds

Bioactive Compounds synthesized by plants that have the potential to be used by humans for a variety of applications. (Biesalski *et al.,* 2009). According to Shahidi (2009), functional foods are defined as those foods which are consumed as part of a usual diet but known to demonstrate beneficial physiological function in regular consumption beyond their nutritional properties. Nutraceuticals are those products derived from the foods, carrying a concentrated amount of a reputed bioactive agent of a food and used in the form of medicinal products (non-food matrix) such as pills, capsules, powders and solutions rendering physiological benefits in human health at higher dosage than that obtained from normal foods (Shahidi, 2007; Shahidi, 2009; Bernal *et al.,* 2010). The main aim of either functional foods or nutraceuticals is to improve health status through the prevention of degenerative diseases. Nearly one third of these diseases is related to the life-style of individuals and thereby tremendously reduces the burden cost of health care (Shahidi, 2007; Shahidi, 2009).

The plant secondary metabolites ingested into the body as pharmaceutical products and their biological activity are effective in the long term. These phytochemicals associated with foods and known for their biological activities include glucosinolates, terpenoids (carotenoids, monoterpenes, and phytosterols) and polyphenols (anthocyanins, flavones, flavan-3-ols, isoflavones, stilbenoids, phenolic acids etc.) (Espín *et al.,* 2007).

Many factors may effects the efficiency of extracting phenolics from plants. Naczk and Shahidi (2004) indicated some factors that could influence the process

such as the chemical nature of the compound, extraction method, sample particle size, storage time and conditions and presence of interfering substances. However, no method or procedure is totally perfect for extracting all or specific phenolics from plants as the compound solubility is governed by the type of solvent used (solubility), degree of polymerization of phenolics and interaction of phenolics with other components that may result in the formation of insoluble complexes (Naczk & Shahidi, 2004).

2.2.3 Cytotoxic effects

Studies by Tandon and Gupta (2004) on the histomorphological effect of *Vitex negundo* extracts in rats indicated that their stomach tissues were not impacted even by toxic doses but there were indications of dose-dependent changes in the tissues of organs such as the heart, liver and lung. The cytotoxic effect of leaf extracts of *Vitex negundo* was tested and affirmed using COLO-320 tumour cells (Smit *et al.*, 1995). In their work, Diaz *et al.* (2003) found the chloroform extracts of *Vitex negundo* leaves to be toxic to a human cancer cell line panel such as Col2 (human colon cancer), hTERT-RPE1 (human telomerase reverse transcriptase-retinal pigment epithelial), HUVEC (human umbilical vein endothelial), KB (human oral epidermoid carcinoma), LNCaP (human hormone-dependent prostate cancer) and Lu1 (human lung cancer) but somewhat in contrast to their findings, Yunos *et al.* (2005) reported that *Vitex negundo* extracts had a non-cytotoxic effect on mammary and genito-urinary cells of mice.

2.2.4 Drug potentiating ability

Various studies have documented that the administration of *Vitex negundo* extracts potentiated the effect of commonly used anti-inflammatory drugs such as ibuprofen and phenylbutazone (Tandon & Gupta, 2006). According to the other documents report that *Vitex negundo* extracts have potential as analgesics such as meperidine, aspirin (Tandon & Gupta, 2005), morphine and pethidine; sedative-hypnotic drugs like pentobarbitone, diazepam (Gupta *et al.*, 1997) and chlorpromazine (Gupta *et al.*, 1999); anti-convulsive agents such diphenylhydantoin and valporic acid (Tandon & Gupta, 2005).

2.2.5 Biological activities

Numerous phytochemical secondary metabolites that provide a wide range of medicinal uses can be found in all parts of the plant, from root to fruit. It is particularly noteworthy that a single plant species is applicable in treating a variety of ailments and disorders in traditional and folk medicine (Vishwanathan & Basavaraju, 2010). Furthermore, some of these applications have been experimentally validated. Utilizing techniques such as cell and tissue culture can be an avenue for rapid propagation and conservation of the plant species and, increase the possibilities of enhancing the quality and quantity of the bioactive secondary metabolites present in the plant (Vishwanathan & Basavaraju, 2010).

It is common knowledge that plants produce various compounds that are defensive mechanisms against microbes and herbivores (Wink, 2004). In line with

this, it can be stated that the extracts of *Vitex negundo* possess inhibitory, lethal activity on biological agents that cause disease and damage to other organisms (Vishwanathan & Basavaraju, 2010). However, interest in the biochemically active ingredients and medicinal properties of *Vitex negundo* has elicited queries on the exact effect or potency of the plant's extracts on other biological organisms. Table 2.4 shows the biological activities of *Vitex negundo*.

Table 2.4 Biological activities of *Vitex negundo* (Vishwanathan & Basavaraju, 2010).

Activity	Action Against
Anti-bacterial	*Escherichia coli, Klebsiella aerogenes, Proteus vulgaris*, & *Pseudomonas aerogenes* (Bacteria)
Anti-feedant	*Spodoptera litura* (Asian army-worm) *Achoea janata* (Castor semi-looper)
Anti-filarial	*Brugia malayi* (Microfilarial parasite)
Anti-fungal	*Alternaria alternata, Curvularia lunata Trichophyton mentagrophytes, Cryptococcus neoformans Aspergillus niger, Candida albicans*
Anti-larval	*Cnaphalocrocis medinalis* (Rice leaf-folder)
Anti-viral	*Plasmodium falciparum* (Virus)
Insecticidal	*Callosobruchus maculatus* (Pulse beetle)
	Phthorimaea operculella (Potato-tuber moth) *Sitotroga cerealella* (Angoumois grain moth) *Aphis citricola* (Spirea aphid), *Aphis gossypii* (Melon or Cotton aphid), *Myzus persicae* (Green peach aphid)
Larvicidal	*Anopheles subpictus,* *Culex tritaeniorhynchus* (Mosquitoes) *Culex quinquefasciatus* (Mosquito)
	Anopheles stephensi (Mosquitoes)
	Plutella xylostella (Diamond-back moth)
Mosquito repellant	*Culex tritaeniorhynchus* (Mosquito)
Mosquito repellant	*Aedes aegypti* (Mosquito)

2.3 Commercial Product and Uses

The whole plant can be said to be useful for medicinal purposes. Starting from the leaves, these are astringent, febrifuge, sedative, tonic and vermifuge and used in diffusing swellings of the joints from acute rheumatism. Further, the leaves produce a juice that is utilized for removing worms from ulcers. At the same time, oil derived from the leaf juice is used for the treatment of sinuses and scrofulous sores. The dried fruit is used in the treatment of angina, colds, coughs, rheumatic difficulties etc (Rastogi *et al.*, 2010).

As a consequence of the effectiveness of *Vitex negundo* for medicinal uses and the treatment of health conditions, several products have been taken with commercial aims from this plant. Details of such commercially available products supported by scientific publications are presented in Table 2.5.

Table 2.5 Commercially products contains ingredients of *Vitex negundo* (Vishwanathan and Basavaraju, 2010)

Manufacturer	Name of product	Used in
Himalaya Drug Co., Bangalore, India	Antiseptic Cream	Wounds, Burns, Fungal skin infections
	Dental Cream	Tooth ache, Bleeding gums
	Himcolin Gel	Erectile dysfunction
Himalaya Drug Co., Bangalore	Acne-n-Pimple Cream	Acne and skin eruptions
	JointCare B cream	Rheumatic disorders
	Muscle and Joint Rub	Muscle strains, musculoskeletal disorders
	Pilex tablet and cream	Haemorrhoids (Piles)
	Rumalaya gel and tablets	Inflammatory musculoskeletal disorders

	V-Gel	Vaginitis, Cervicitis
Surya Herbal Ltd., Noida, India	Relief Cream	Joint and Muscle pain, Stiff back
	Rheumanaad Tablet and Cream	Rheumatic Pain, Sprain
	Ostranil Gel	Osteoarthritis, Lumbago
Ambica Research & Development Pvt. Ltd, New Delhi, India	Amgesic Arthritis Tablets	Arthritis
IndSwift Ltd., Chandigarh, India	Arthrill Capsules and Massage oil	Arthritis, Joint pain, Frozen shoulder, Gout, Cervical spondylitis
Hamdard Laboratories, New Delhi, India	Jigrine	Liver ailments
Dey's Medical, Kolkata, India	Itone Eye Drops	Eye ailments

2.4 Botany of *Clitoria ternatea* Species

Clitoria ternatea is a highly nutritious growing legume plant belongs to the family Fabaceae, sub family papilonaceae and possesses a number of recognized names, such as *Clitoria purpurea, Clitoria ternatensum* and *Ternatea ternatea.* However,

Clitoria ternatea (CT) and *Clitoria purpurea* are partially domesticated and may have potential for forage use (Gomez and Kalamani, 2003).

The vine of this plant are climbing, herbaceous, tall and slender with five leaflets while the flower colour ranges from white to blue with white or yellowish canter. The length of the leaves range from 4 to 10 cm, and the leaflets can be from 2 to 7 cm long (Goh *et al.,* 1995). Morton (1981) described the plant with an ornamental perennial climber, more than 2–3 m in height, growing in gardens and also wild, holding conspicuous white or blue flowers with conch-shell shape.

Firstly, the plant is originated from America, but nowadays it can be cultivated and naturalize throughout the humid tropics below 1600 m elevation. The plant reaches widespread occurrence in tropical Asian countries rather than India, Philippines. It has also seen widely in South and Central America, the Caribbean and Madagascar (Anonymous, 1988; Sivarajan & Balachandran, 1994). In U.S.A, the plant species grows widely from Florida to Texas and from New Jersey to Kentucky and Arkansas. Also, it could be found in several Latin American and West Indies countries such as Mexico (Sonora and Tamaulipas south), Bahamas, Cuba, Dominican Republic, Haiti, Jamaica, Puerto Rico, Turks and Caicos Islands, several Virgin and Leeward Islands, and in Paraguay and Argentina (Austin & Honychurch, 2004). The scientific name of the genus is taken from Greek word, a spur, prickle, sharp point, the center, and sema, a signal, referring to the spurred standard petal (Austin & Honychurch, 2004). The classification under *Centrosema* or *Bradburya* was at some point an object of debate especially as similarities exist between species of *Clitoria and Centrosema*. A distinguishing characteristic of both the *Centrosema*

and *Clitoria* are flowers rotated at 180^{o} with the banner pointing downwards (Mukherjee *et al.*, 2008).

The pods are 5–10 cm flat, long, mostly straight with 6–11 seeded and sharply beaked (Karandikar & Satakopan, 1959; Pillai, 1976). Seeds found as yellowish-brown or blackish appearance with an oval or subglobose shape. Common names for *Clitoria ternatea* (CT) include butterfly pea, conch flower, shankapushpi, Aparajit (Hindi), Aparajita (Bengali), and Kakkattan (Tamil). According to Mukherjee *et al.* (2008), the origin of the plant is traceable to the Caribbean, México and Central America and later was divided to the Indian subcontinent. *Clitoria ternatea* has now been commercialized as an ornamental, fodder and medicinal plant. Due to its attractive blue colour, which can be obtained by soaking the plant in water, the flower of the plant has widespread uses in Malaysia (figure 2.3).

Figure 2.3 The flower of *Clitoria ternatea*

It provides food colouring for local cuisine's such as *Nasi Kerabu*, which is very popular in Kelantan (figure 2.4). *Nasi kerabu* is a traditional Malaysian dish that is famous for its blue rice. In addition to the rice, this filling dish also contains fish, which is the base of the kerabu, or vegetable mixture. Table 2.6 shows the taxonomic classification of *Clitoria ternatea*.

Figure 2.4 Nasi Kerabu

Table 2.6 Taxonomic classification of *Clitoria ternatea* (United States Department of Agriculture, 2010).

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Clitoria L.*
Species	*Clitoria ternatea L.*

2.4.1 Chemical constituents

High amounts of growth substances such as indole acetic acid, kinetin and gibberelic acid can be found in the roots of *Clitoria ternatea* (Yadava & Verma, 2003). The roots of *Clitoria ternatea* also contain antimicrobial flavonol; glycoside 3,5,4'-trihydroxy-7-methoxyflavonol-3-O-β-D-glucopyranoside (Yadava & Verma, 2003).