THE EXTRACTION OF ANTHOCYANIN FROM *CLITORIA TERNATEA* (BLUE PEA FLOWER) BY USING SPRAY DRYER

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ABSTRACT

Nowadays, interest in anthocyanin pigments shows an increasing tendency due to their possible health benefits as antioxidants, anti-inflammatory, anti-viral, protection from cardiovascular damage, diabetes preventation and vision improvement. *Clitoria ternatea*, a local flower which also an indigenous climber herbs, has been found to produce pigments mostly anthocyanin. In this research, anthocyanin from *Clitoria ternatea,* also known as blue pea flower was extracted by using water. The anthocyanin extract were encapsulated by using spray dry method, which three parameters are being studied, that are inlet air temperature, maltodextrin addition, and also feed flow rate. The technique of using spray dryer is preferable as transformation of juices into dry powders is quite hard due to the high sugar and acid contents. The experimental research was done by using Laboratory Scale Spray Dryer SD06AG, with the range of 160°C to 190°C of temperature, maltodextrin addition of 0% to 40% and feed flow rate of 5 ml/min to 20 ml/min. Further analysis on the quality of the powder obtained from spray drying process were verified by measuring total phenol content, anthocyanin content and antioxidant activity by using UV-Vis U 1800 Spectrophotometer at certain wavelengths. Maltodextrin DE10 had been used as carrier in the spray dryer to prevent wall-deposition formation during the process. As result indicates that at 170°C of inlet temperature, with 30 % maltodextrin addition and 10 ml/min of feed flow rate is the best condition to encapsulate CTAE by using spray dry.

PENGEKSTRAKAN ANTOSIANIN DARIPADA *CLITORIA TERNATEA* (BUNGA TELANG) MENGGUNAKAN PENYEMBUR-KERING.

ABSTRAK

Kini, kepentingan terhadap pigmen antosianin menunjukkan kecenderungan yang semakin meningkat disebabkan oleh manfaat kesihatannya sebagai antioksidan, anti radang, anti virus, pencegah kerosakan jantung dan kencing manis, dan untuk meningkatkan daya penglihatan. *Clitoria ternatea*, sejenis bunga tempatan yang juga merupakan herba yang memanjat, telah diketahui kebolehannya untuk menghasilkan pigmen yang kebanyakannya adalah antosianin. Dalam kajian ini, antosianin dari *Clitoria ternatea* yang turut dikenali sebagai bunga telang, telah diekstrak dengan menggunakan air. Proses pengkapsulan antosianin yang terkandung di dalam bunga telang dilakukan menggunakan penyembur kering, di mana tiga parameter telah disiasat, iaitu suhu awal, peratusan tambahan maltodextrin dan halaju kemasukan suapan. Teknik menggunakan penyembur kering adalah lebih baik kerana proses transformasi daripada jus ke bentuk serbuk adalah sukar berikutan kewujudan gula dan asid yang tinggi di dalamnya. Eksperimen penyelidikan ini telah dijalankan dengan menggunakan Penyembur Kering Skala Makmal SD06AG dengan julat suhu daripada 160°C ke 190°C, 0% hingga 40% penambahan maltodextrin dan julat halaju kemasukan suapan dari 5 ml/min ke 20 ml/min. Analisis lanjut terhadap kualiti serbuk yang diperolehi daripada proses semburan kering telah disahkan dengan mengukur jumlah kandungan fenol, kandungan antosianin dan aktiviti antioksidan, menggunakan spektrofotometer UV-Vis U 1800 pada panjang gelombang yang tertentu. Maltodextrin DE10 telah digunakan sebagai agen pembawa dalam penyembur kering untuk menghalang pembentukan plak pemendapan di dinding kebuk. Hasil eksperimen menunjukkan pada keadaan suhu awal 170°C, 30% tambahan maltodextrin pada halaju kemasukan suapan sebanyak 10 ml/min adalah yang terbaik untuk pengurungan ekstrak antosianin Clitoria ternatea menggunakan penyembur kering.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, the world is inclusively focusing on enhancing human health from time to time. The science communities are work hard on the development of medicines and disease treatments, especially the improvement from the leaves, flowers and any edible plants. Plant has been used as source of medicine to treat various ailment of human since the ancient times, especially local plant.

Aligned with the bombardment of public concerns on the usage of synthetic addition in food among the consumers and industries, Montes et al. (2005) stated that there is a growing interest in the food industry for new viable sources as alternatives for the food colorants. Not only being used in food, natural colorant is also use in cosmetics, pharmaceuticals, fabrics, paints, toys and many more. The safety for the consumption is now being a significant issue due to the toxicity and allergic effect of synthetic dyes, to human and environment (Rosmawati et al., 2010).

Clitoria ternatea is one of local plant with medicinal properties that can be found widely in tropical countries. This edible flower had been used to dye rice cake

in Malaysia and being eaten as vegetables in India and Philippines. This flower is also being used traditionally as remedy for diuretic, anthelmintic, rheumatism, bronchitis, purgative, urinogenital disorder, demulcent and anticancer (Patil & Patil, 2011).

For nearly ten years ago, genetically-modified, color-altered varieties of the important flower crops have been commercially exposed. Those flower crops are producing pigments, that are mostly anthocyanin, a most wide range group of flower pigments, that is reported to be from yellow to blue. This group is a main colorant molecules that are derivatives of basis classes such as pelargonidin for orange-red color, cyanidin for red hues and delphinidin for lilac to blue hues (Vankar & Srivasta, 2010).

Anthocyanin which is a type of bioactive compounds has been studied to have strong antioxidant activity as cited in Zhang et al.(2011). Corresponding to the antioxidant activity, it is also stated to have higher total phenol content, as anthocyanin are belongs to flavonoids, a phenolic compound that is major antioxidants of our diet.

The growing demands of anthocyanin due to their beneficial health effects and the beautiful colorants to food system were proved by their contribution on treatment of liver disfunction, hypertension, vision disorders, microbial infections and diarrhea and also dietary supplement (Zhendong et al., 2010).

“Main minerals and essential trace elements are very important in biological processes, and play a vital role in normal growth and development and have also been involved in the prevention of some chronic diseases” (Gorinstein et al., as cited in Henriquez, 2010).

According to Coralia et al., (2010), the spray dryer is preferable technique as transformation of fruit juices into dry powders become a key challenges due to their high sugar and acid contents. Therefore, spray dryer is chosen as it capability of atomizing liquid product to form a firm powder. In place of minimizing the economical costing for preservation of natural colorants by using coating material to ensnare the ingredients, Ersus and Yurdagel (2006) claimed that encapsulation by using spray dry is a better alternative to be applied commercially.

Spray dryer is frequently correlated to wall-deposition problems. Therefore, carrier agent is added to reduce the powder stickiness due to the low glass transition temperature (Tg) of the low molecular weight sugars present in the products. There are several types of carrier agent that had been used in the industry, such as Arabic gum, maltodextrin, and starch. Maltodextrin is functional for better microencapsulation, which able to protect sensitive food components against unfavourable ambient conditions, flavours and aromas preservations, volatility reduction, and better appearance of products (Phisut, 2012).

1.2 Problem Statement

Many of the research had been done for extracting the anthocyanin from plants to the maximum as the great potential in enhancing human health, despite of the capability to give natural color for food engineering, thus meet demand in the industries. The worldwide demand for anthocyanin from black grapes alone is estimated to be 10 000 tonnes annually. Therefore, an increasing number of studies on other possible plants such as *Canna indica*, *Clitoria ternatea*, *Delonix regia*,

Hibiscus mutabilis, *Punica granatum*, and many more had been done during past two to three decades.

Clitoria ternatea had been implicated to have medicine properties and contain antioxidant properties. *Clitoria ternatea* has its own advantage as the plant is easy to grow in Malaysia weather which may lead to high productivity of the anthocyanin. There are several researches done before on *Clitoria ternatea* which evaluating this flower to contain higher anthocyanin index compared to other flowers, with extra specialties such as great abundance in Malaysia, growth conditions and presence of variety of chemical constituents.

With a view to prolonging the shelf life of a product and at the same time having a considerable reduction in volume, dehydration of juices or suspension into powdered particles is an effective technique. There are various types of drying method, freeze drying, spray drying and tunnel drying. Take into account of economical capacity, spray dry had been proven to be foremost method to be commercialized in the industry, compared to other drying method.

Spray dry has its own advantages, as it can be applied to both heat resistance and heat sensitive products. In addition, the continuous process implemented in spray dry provides better appearance of product, which can be powders, granules or agglomerates, depending upon the physical and chemical properties of the feed.

Critical parameters of spray drying are identified, such as inlet and outlet temperature, feed flow rate, types of carrier agent, viscosity, solid content, surface tension, and nozzle material. These parameters influence to the process pattern, which may lead to changes in physical properties of products.

1.3 Research Objective

The main objective of this research is to microencapsulate anthocyanin extract from *Clitoria ternatea* (blue pea flower) by using spray dryer.

The evaluable objectives of this research are:

i. To investigate the effect of inlet temperature, maltodextrin addition and feed flow rate during the spray dry process of CTAE.

ii. To identify the powder appearance and product yield from the spray drying process.

iii. To determine the total phenol content, anthocyanin content and antioxidant activity of powdered product from spray dry process.

1.4 Scope of Study

To achieve the objectives, few scopes have been identified in this research: As this research is focusing on application of spray dryer, three parameters are investigated. They are inlet temperature, maltodextrin addition and feed flow rate. The *Clitoria ternatea* Anthocyanin Extract (CTAE) undergoes spray drying process in several conditions. Four different inlet temperatures had been studied, that are 160°C, 170°C, 180°C and 190°C. Besides that, percentage maltodextrin addition of 0%, 20%, 30% and 40% are also had been applied separately to the CTAE. The effect of four different feed flow rates of 5 ml/min, 10 ml/min, 15 ml/min and 20 ml/min are also considered in this research.

1.5 Rational and Significance

The aim for this study is to explore for new valuable source of anthocyanin from local flower, *Clitoria ternatea* or formerly known as blue pea flower. As the flower is easily grown in Malaysia, is important and kind a beneficial to be established as new findings with lot of great things. Technically, in the context of economy, there will be reduction in growth costing and maintenance. Besides that, it is also to extract anthocyanin from this edible flower by using spray drier technique. Nowadays, spray dry is used widely in the industrial scale as it is found to have more economical profits. On the other hand, this technique provides better preservation of the flavor, ingredients and aroma of the products. After that, analysis of the total phenol content, antioxidant activity and anthocyanin content of each samples of spray dry done in order to determine the available contents. The capability of this local flower to produce great amount anthocyanin, an antioxidant with positive health effect can be introduced and commercialized into local industries.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The chemistry, biochemistry and molecular biology of the biosynthesis of flavonoids are identified, that is the largest and important group of flower color pigments is anthocyanin. It is a subclass of flavonoids which not only functional as coloring agents, but also contain an array of health-promoting benefits.

Nowadays, there are numbers of research done in order to identify flavonoids present in fruits and plants such as sweet potato, berries, hibiscus, and many more due to their potential-promoting effects, besides the technological reasons and organoleptic properties.

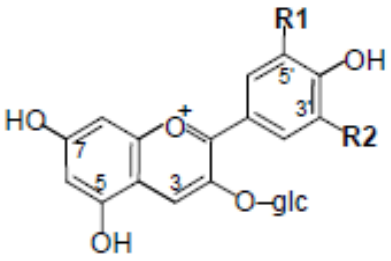
Numerous publications had identified various methods used for the extraction and storage condition to maintain the stability, as well as reducing the production cost. Freeze drying, drum drying, spray drying, tunnel drying are examples of drying method had been used in order to convert the juice into powder, to extending the shelflife, compared being left in suspension.

2.2 Anthocyanin

Derived from Greek words *anthos* (flower) and kyanos (blue), the word anthocyanin was inventively used to express the blue pigments of cornflower, *Centura cyanus* (Marquart, as cited in Jordheim, 2007). Anthocyanin is an antioxidant eaten in large amounts by primitive human that capable of slowing or preventing the oxidation of other molecules. They are of strongest physiological effects of any plant compound. Apart from that, they are also things of beauty as their capability to produce pigments for pansies, petunias and plums. Anthocyanin that can be found in roots, caudexes, leaves, flowers and also fruits, apply a role as substitutes for synthetic pigments as they have the physiological functionality (Zhendong et al., 2010).

2.2.1 Structure and Characteristics

Anthocyanin are glycosides which consist of an aglycone called anthocyanidin (Figure 2.1) that linked to sugars (Figure 2.2) and many cases, acyl group (Figure 2.3). Anthocyanidin is unstable to light and insoluble in water, leading to no occurance in the free state. The linkage between anthocyanidin and sugars provides better stability and water solubility. With structure of $C_6C_3C_6$ skeleton, anthocyanin are positively charged at acidic pH and this equilibrium form is called flavylium cation (2-phenylbenzopyrylium). At some condition, anthocyanin can be differed to each other by glycoslyation of hydroxyl group, nature of glycosyl units, substitution pattern and potential aliphatic and aromatic acylation.



R1	R2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OH	OCH_3	Petunidin
OCH_3	H	Peonidin
OCH_3	OCH_3	Malvidin

Figure 2.1 Structure of anthocyanidins (aglycone).

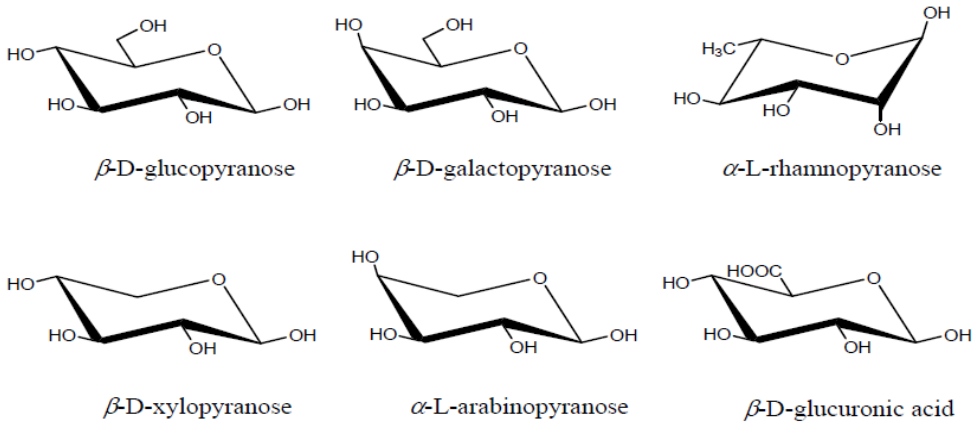


Figure 2.2 Monosaccharides found in anthocyanin structures.

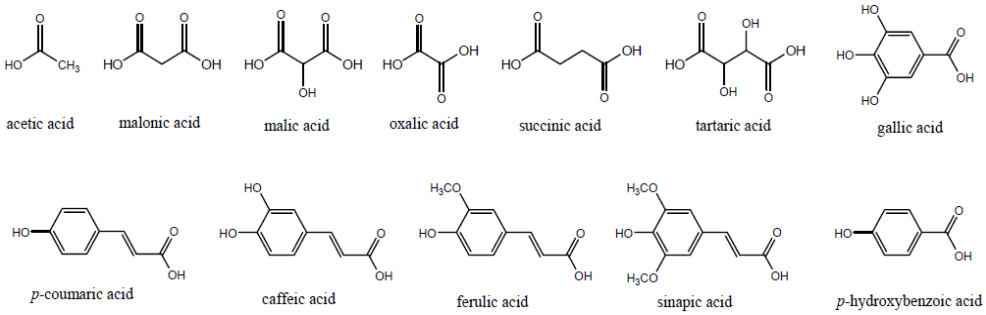


Figure 2.3 Structures of acyl substituents found in anthocyanin.

As mention before, the characteristic of anthocyanin is that it is water soluble glycosides of polyhydroxyl and polymethoxyl derivatives of 2-phenylbenzopyrylium or flavylium salts. According to Ghosh and Konishi (2007), most common anthocyanidin that can be found in anthocyanin are pelargonidin, cyanidin,

delphinidin, peonidin, petunidin, and malvidin which are named according to the number and position of hydroxyl group of the flavan nucleus. Anthocyanin stability is increased with the presence of acylation of the sugar residues with cinnamic or aliphatic acids. Cyanidin had been known to be most anthocyanin found in edible parts of plants, leading among pelargonidin, peonidin, delphinidin, petunidin, and malvidin.

Anthocyanin as a phenolic compound and non-toxic pigment presents a spectrum from orange to blue in color in the natural world, which satisfies the consumer needed in food colors. This phenol structure capability of capturing free-radicals, to play a role as antioxidant are reported to be higher than vitamin C and E (Padma et al., 2010). Their hue and structure are relying on pH and presence of co-pigments. The same anthocyanin may have different colors in different pH condition, depending on the pH of the organelle. For pH condition, at pH 1-3 the flavylium cation is red, and colourless at pH 5. When it comes to pH 7-8, the blue purple quinodial base is present. Cyanidin, delphidinin, and pelargonnidin are three non-methylated anthocyanidin, which have most abundant glycosidesin nature; in pigmented leaves (80%), 69% in fruits and 50% in flowers.

The extraction of anthocyanin from plant or fruits currently employed using methanol, ethanol, acetone, water or mixtures as solvents. However, the stability of these anthocynin is affected by the structural modifications with hydroxyl, methoxyl, glycosyl, and acyl groups. Not to forget, also by environmental factors such as temperature and light. Therefore, anthocyanin experience color decline during storage and process.

2.2.2 Diverse Health Effects

Studies on anthocyanin from plant and fruits had been intensified from the past two or three decades due to the potential health effects, and now they are conferred as important nutraceuticals. Anthocyanin display a wide range of biological activities, which includes antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic activities; vision improvement, anti-diabetic, stomach ulser and also reducing heart disease.

Powerful antioxidant like anthocyanin are capable to reduce radical scavenger activity by glycosylation of an anthocyanin, compared with aglycone as it decrease the ability of anthocyanin radical to delocalize electrons.

During inflammation, enzymes cause connective tissue damages in capillaries, thus causing blood leaking into surrounding tissue. Anthocyanin neutralize enzymes that destroy the connective tissue. The antioxidant capacity inside prevents oxidants from continuing damage the connective tissue. Later, anthocyanin is responsible in damaged blood vessel walls treatment (Sterling, 2001).

In ancient years, the anthocyanin of bilberries has been used for improving visual acuity and treating circulatory disorders. In the study done in French at 1964, a case study experiment on 36 people held to investigate their capability to adapt light or dark both before and after taking bilberries anthocyanin. Few hours after the supplementation, they have better eyesight significantly, although the effect wore off within 24 hours.

Diabetic caused by complication of microvessel damage from high sugar content in the blood. The consequence of linkage formation between collagen proteins with sugars is abnormal polymeric blood vessel collagen. Sterling (2001)

claimed that in German, 12 adults with diabetic disease consult 600 mg of anthocyanin daily for two months. Their gum tissue samples were taken before and after the consultation. The result showed decreasing abnormal collagen production after the supplementation.

Traditionally, anthocyanin from bilberries were used for treating ulcers and increase of stomach mucus production to prevent stomach from injury. Besides that, anthocyanin of delphinidin is capable to inhibit epidermal growth factor receptor in cancel cells compared to malvidin.

2.2.3 Anthocyanin Content of Common Plants

Anthocyanin is one of food bioactive compound with a double interest, one technological, due tot heir impact on the sensorial characteristics of food products.Studies had been done on plants with anthocyanin such as blue and red flowers, purple corn *(Zea mays* L.*)*, black carrot *(Daucurcorata* L.*)* and also mulberry. But, there is a need to identify the existence of anthocyanin in our local flower as they are widely grown in Malaysia. They are *Ixora chinensis, Hibiscus mutabilis, Nerium oleander, Thunbergia erecta, Rosa indica, Ixora chinensis, Ruellia tuberose* and many more. Several structural studies of anthocyanin from *Clitoria ternatea* had been reported, but it is still limited.

Table 2.1 Total anthocyanin content in red and blue flower.

No	Latin Binomial	Common Name	Family	Color	Total Anthocyanin Content (mg/kg)
1.	*Lagerstroemia indica*	Sawani	*Lythraceace*	Violet	36.22
2.	*Hibiscus mutabilis*	Gulzuba	*Malvaceae*	Pink	52.94
3.	*Delonix regia*	Gulmohar	*Fabaceae*	Orange	101.13
4.	*Mirabilis jalapa*	4 o'clock	*Nyctaginaceae*	Magenta	338.61
5.	*Impatients balsamina*	Balsam	*Balsaminaceae*	Red	336.56
6.	*Jathropha integerrima*	Bhagirend	*Euphorbiaceae*	Red	152.82
7.	*Portulaca graniflora*	9 o'clock	*Portulacaceae*	Red	131.96
8.	*Canna indica*	Keli	*Cannaceae*	Red	96.29
9.	*Quisqalis indica*	Madhumalti	*Combretaceae*	Red	72.30
10.	*Rosa indica*	Rose	*Rosaceae*	Red	64.52
11.	*Nerium oleander*	Kaner	*Pocynaceae*	Red	55.94
12.	*Ixora chinensis*	Rangan	*Rubiaceae*	Red	50.84
13.	*Thunbergia erecta*	Bush clock vine	*Acanthaceae*	Blue	46.95
14.	*Ruellia tuberose*	Ruellia	*Acanthaceae*	Blue	46.84
15.	*Clitoria ternatea*	Aparajita	*Fabaceae*	Blue	227.42

(Source: Vankar and Srivastava : Anthocyanin Content in Red and Blue Flower)

From research done by Vankar and Srivastava (2010), the highest value of TAC was found in *Mirabilis jalapa*, followed by *Impatients balsamina, Clitoria ternatea, Jatropha integerrima, Portulaca graniflora, Delonix regia, Canna indica, Quisqalis indica, Rosa indica, Nerium oleander, Hibiscus mutabilis, Ixora chinensis, Ruellia tuberose, Thunbergia erecta, and Lagerstroemia indica*.

Research done by Fan et al., (2007) on anthocyanin extract form purple sweet potato *(Ipomoea batatas)* reported that anthocyanin existed in the fruit are mono- or di-acylated forms of cyaniding and peonidin

2.3 *Clitoria ternatea* (Blue Pea Flower)

The words *'Clitoria'* is taken describing the flowers appearance, the vine shape of human female pudenda. Thus, the Latin name of the genus '*Clitoria'* from 'clitoris'.

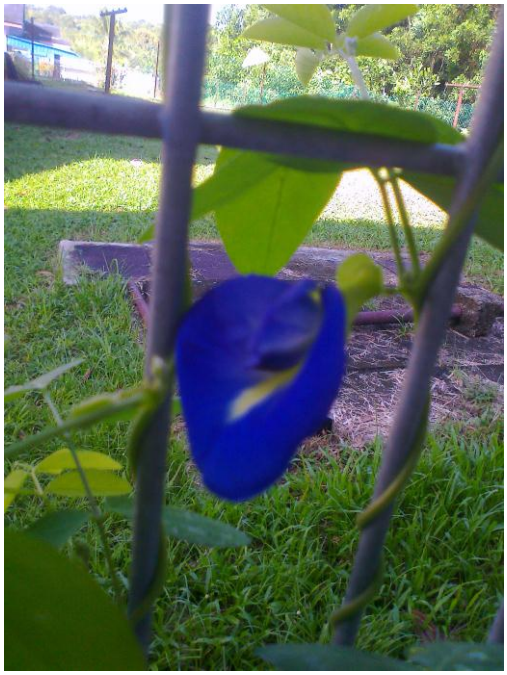


Figure 2.4 Blue Pea Flower

Clitoria ternatea is a perennial climbing herb that can be found abundantly in tropical equatorial Asia, but had been introduce to Africa, Australia and America includes Angola, Benin, Burundi, Cabinda, Cameroon, Cape Verde Is, Chas, Ethiopia, Malawi, India, Sudan and area of Indian Ocean. In Bahasa, this plant is known as 'bunga telang', 'blue pea flower, butterfly pea flower, pigeon wings, tropical alfalfa, mussel-shell climber (English) and Aparajita in Bangladesh and Sankupushpam in Kerala. This plant has two varieties of colors, white and blue petals colors.

2.3.1 Family

The world-wide health organizations are now curating some important nutraceutical and medicinal plant species. One of them is *Clitoria ternatea*, a plant with medicinal behavior that being used since ancient times.

Clitoria ternatea, a species belongs to *Fabaceae* family which is also placed in *Papilionaceae* family, exhibits concentrated cobalt blue color in botanical field. It has been used traditionally to treat various diseases such as urinogenital disorder, bronchitis, purgative, rheumatism and diuratic (Patil & Patil, 2011).

Table 2.2 Scientific classification of blue pea flower

Scientific Classification	
Kingdom	Plantae
Divisions	Spermatophyte
Subdivision	Angiospermae
Class	Fudicots
Subclass	Rosids
Order	Fabales
Family	Fabaceae/Leguminosae
Subfamily	Faboideae/Papilionoideae
Tribe	Phaseoleae
Subtribe	Clitoriinae
Genus	Clitoria
Species	*Clitoria ternatea* L.