IDENTIFICATION OF VOLATILE AND NON-VOLATILE COMPOUNDS OF CORNSILK AND ITS ASSOCIATION WITH DIURETIC PROPERTIES IN RATS

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by

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Abbreviations

ANOVA	:	Analysis of Variance
ARASC	:	Animal Research and Service Centre
CAE	:	Corn silk aqueous extract
CCRD	:	Central Composite Rotatable Design
CME	:	Corn silk methanolic extract
CSP	:	Cornsilk powder
DCM	:	Dichloromethane
ED_{50}	:	Effective dose of 50 % stimulation
$FeCl_3$	:	Ferum chloride
GC-MS	:	Gas-Chromatography Mass-Spectrometry
HCl	:	Hydrochloric acid
H_2SO_4	:	Sulphuric acid
MOA	:	Ministry of Agriculture Malaysia
MOH	:	Ministry of Health Malaysia
MUPA	:	Makmal Unit Perkhidmatan Analisis
MW	:	Molecular weight
pED_{50}	:	pharmacological effective dose of 50 % stimulation
SD	:	Sprague - Dawley
VC(s)	:	Volatile compound(s)

Symbols

g	:	gram
h	:	hour

kg	:	kilogram
min	:	min
ml	:	millilitre
mg	:	milligram
mmol/l	:	millimol/ liter
mosmol/kg	:	milliosmol/ kg
wt.	:	weight
ºC	:	degree celcius

PENGENALPASTIAN KOMPONEN MERUAP DAN BUKAN MERUAP DALAM SUTERA JAGUNG DAN KAITANNYA DENGAN CIRI-CIRI DIURETIK PADA TIKUS

ABSTRAK

Pengenalpastian komponen meruap dan bukan meruap dalam sutera jagung dan kaitannya dengan ciri-ciri diuretik telah dikaji. Serbuk sutera jagung dipanaskan pada sepuluh kombinasi masa dan suhu yang berbeza termasuk satu serbuk sutera jagung kawalan (tanpa pemanasan) bagi mengenalpasti komponen meruap menggunakan GC-MS. Dua jenis ekstrak sutera jagung yang digunakan dalam kajian ini iaitu akues dan metanolik bagi mengenalpasti komponen meruap dan bukan meruap menggunakan GC-MS dan ujian penskrinan. Setiap ekstrak, diberikan kepada setiap tikus Sprague-Dawley secara oral bagi penentuan ciri-ciri diuretik. Selepas 24 jam pemberian ekstrak, paras pengeluaran urin, pH, osmolaliti, Na^+, K^+ and Cl^- ditentukan. Parameter yang sama diperhatikan untuk empat hari yang seterusnya bagi mengkaji kesan dos berulang terhadap ciri-ciri diuretik. Beberapa sebatian fitokimia telah dikenalpasti dalam ekstrak akues dan metanolik sutera jagung melalui kaedah penskrinan iaitu flavanoid, fenol, tannin, plobatannin, alkaloid, saponin dan kardiak-glikosida termasuk juga terpenoid di dalam ekstrak metanolik. Selain itu, tiga belas sebatian meruap telah dikenalpasti dalam ekstrak akues melalui analisis GC-MS termasuklah cis-2-pentena, asid heksadekanoik, pyranone, 2-metilheptana, etil cyclopentana, furfural, metil-5-furfural, asid asetik, lakton G, 1-naftol dan tetrahidro-3-furanol. Ekstrak metanolik mengandungi empat sebatian meruap iaitu 5-hidroksimetil-furfural, 4-metil itaconate, asid asetik dan asid lakton 3-deoksi-d-mannoik. Rawatan pra-pemanasan serbuk sutera jagung ini

menghasilkan pelbagai aroma yang unik kesan daripada interaksi beberapa sebatian meruap. Pra-pemanasan serbuk sutera jagung pada 110 °C (15 min) menghasilkan bau bungaan yang sangat menarik berbanding rawatan yang lain kemungkinan disebabkan oleh kehadiran benzeneetanol. Sebaliknya, pra-pemanasan serbuk sutera jagung pada 135 °C (20 min) dan 145 °C (15 min) menghasilkan bau karamel yang kuat mungkin disebabkan oleh kehadiran 2-metil, tetrahydrofuran-3-one dan 2-asetilpyrol. Ekstrak akues dan metanolik sutera jagung meningkatkan pengeluaran urin secara signifikan ($p \leq 0.05$) pada dos yang tinggi apabila dibandingkan dengan air suling. Diuresis yang disebabkan oleh ekstrak akues adalah setara dengan chlorothiazide (21.25 ml) dan mannitol (19.56 ml) pada dos 600, 700 dan 800 mg/kg (20.13, 19.63 dan 20.00 ml). Ciri yang sama diperhatikan dalam ekstrak metanolik pada dos 40 dan 60 mg/kg (18.25 dan 18.87 ml). Ekstrak akues dan metanolik sutera jagung meningkatkan pengeluaran Na^+ secara signifikan ($p \leq 0.05$) pada dos 500 – 800 mg/kg serta 15 dan 60 mg/kg. Kesemua dos ekstrak akues tidak mengubah pengeluaran K^+, sementara dos ekstrak metanolik pada 15 dan 60 mg/kg secara signifikan ($p \leq 0.05$) mengurangkan pengeluaran K^+ dalam urin. Kedua-dua ekstrak menunjukan kesan penahanan-kalium diuretik. Tiada perubahan signifikan ($p \leq 0.05$) diperhatikan bagi Cl^-, pH dan osmolaliti selain sedikit kealkalian di dalam urin bagi tikus untuk kedua-dua ekstrak sutera jagung. ED_{50} bagi ekstrak akues dan metanolik sutera jagung dapat diperhatikan pada 454.10 mg/kg dan 16.64 mg/kg. Pemberian oral secara berulang untuk empat hari seterusnya bagi kedua-dua ekstrak pada semua dos tidak menunjukkan nilai perubahan signifikan ($p \leq 0.05$) untuk semua parameter jika dibandingkan dengan hari pertama. Berdasarkan sorotan kajian, terdapat tiga sebatian yang dikenalpasti mempunyai kaitan dengan aktiviti diuretik iaitu pyranone, etil siklopentana dan asid heksadecanoik.

IDENTIFICATION OF VOLATILE AND NON-VOLATILE COMPOUNDS OF CORNSILK AND ITS ASSOCIATION WITH DIURETIC PROPERTIES IN RATS

ABSTRACT

Identification of volatile and non volatile compounds of corn silk and its association with diuretic activity were investigated. Corn silk powder was heated at ten different combinations of time and temperature, including control corn silk powder (unheated) for identification of volatile compounds via GC-MS. Two types of corn silk extracts were used in the present study namely aqueous and methanolic to identify the presence of volatile and non-volatile compound using GC-MS and screening test, respectively. Each extract was orally fed to individual Sprague-Dawley rats for diuretic properties determination. After 24 h administration of extract, the level of urine excretion, pH, osmolality, Na^+, K^+ and Cl^- were measured. The same parameters were observed for another four days to investigate the effect of repeated dosage on diuretic properties. Several phytochemical compounds had been identified in aqueous and methanolic extract of corn silk through screening method which contained flavonoids, phenols, tannins, phlobatannin, alkaloids, saponins and cardiac glycosides including terpenoids in methanolic extract. In addition, thirteen volatile compounds were identified in aqueous extract by gas GC-MS which consists of cis-2-pentenal, hexadecanoic acid, pyranone, 2-methylheptane, ethyl cyclopentane, furfural, methyl-5-furfural, acetic acid, lactone-G, 1-naphtol and tetrahydro-3-furanol. Meanwhile in methanolic extract, four volatile compounds were identified including 5-hydroxymethyl furfural, 4-methyl itaconate, acetic acid and 3-deoxy-d-mannoic acid lactone. These preheating treatments of corn silk

powder resulted in unique aromatic odour due to the interaction of several volatile compounds. Preheating corn silk powder at 110 °C (15 min) was found to produce the most pleasant flowery odour among all treatments may be due to the presence of benzeneethanol. Vice versa, preheating corn silk powder at 135 °C (20 min) and 145 °C (15 min) gave strong caramelized odour, that probably contributed by 2-methyl, tetrahydrofuran-3-one and 2-acetylpyrrole. Aqueous and methanolic extract of corn silk significantly ($p \leq 0.05$) increased urine excretion at higher doses with respect to distilled water. The diuresis induced by aqueous extract was comparable to chlorothiazide and mannitol at doses of 600, 700 and 800 mg/kg (20.13, 19.63 and 20.00 ml, accordingly). Similar trait was observed in methanolic extract at doses of 40 and 60 mg/kg (18.25 and 18.87 ml, respectively). Aqueous and methanolic extract of cornsilk significantly ($p \leq 0.05$) increased Na^+ excretion at doses of 500 – 800 mg/kg and 15 and 60 mg/kg, respectively. All doses of aqueous extract did not alter the excretion of K^+, while methanolic extract doses at 15 and 16 mg/kg significantly ($p \leq 0.05$) decreased the K^+ excretion in the urine. Both extracts showed potassium-sparing diuretic effect. There was no significant ($p \leq 0.05$) alteration observed in chloride, pH and osmolality aside from slight alkalization of urine in rats treated with corn silk extracts. The ED_{50} of aqueous and methanolic extract of corn silk were observed at 454.10 mg/kg and 16.64 mg/kg, respectively. Repeated oral administration for another four consecutive days of both extracts at all doses showed no significant ($p \leq 0.05$) value in all parameters when compared to day 1. Based on literature review, three compounds were identified to have potential association with diuretic activity namely pyranone, ethyl cyclopentane, and hexadecanoic acid.

CHAPTER 1

INTRODUCTION

1.0 Prelude

Corn silk is a hair of a corn. It is known as *Zea Mays* hairs or *Zea mays* stigmata. *Zea mays* L. belongs to a family gramineae. It is originated from North America and called as corn. Later in 1575, after years coming by, *Zea mays* is then found in Asean, more particularly are western China, east India and also Philippine (Gibson and Benson, 2002). *Zea mays* is also known as a different name in different places. As in America it is called as corn, in Philippine it is known as '*jagnog'*. Meanwhile, in Malaysia, *Zea mays* is called as '*jagung'*, while corn silk is known as '*sutera jagung'*.

Whole corn plant consists of several parts with various functions. The major components are corn, corn fruit, corn silk, husk, leaves and stem. Corn kernel from corn fruit is edible to human while cornsilk, husks, leaves and stem are sometimes used as a feedstock or mainly discarded. In Malaysia, corn silk is also thrown away after have been separated from the corn fruits. The elimination of this agricultural by-product is believed to be associated with the lack of knowledge regarding the health benefit of corn silk. Corn silk is well-known to give value to

men's health in other countries like America and China. In addition, corn silk is believed to contain various essential phytonutrients and can significantly heal some illnesses related to kidney, heart and blood pressure. Besides that, corn silk have been used as a functional ingredient in various preparations of foods, cosmetics and pharmaceutical products (Hasanudin *et al.*, 2012).

Corn silk has a yellowish thread-like colour while fresh. After drying, it maintains the yellowness colour intensity and produced a sweety aromatic odour. There are many types of precursors that influence the production of volatile compounds in plant like tea leave (Wang *et al.*, 2008), cocoa bean and pandan (Cheetangdee and Chaiseri, 2006). The production of pleasant aromatic odour is normally caused by the reaction of amino acid and reducing sugar. The development of attractive aroma generated from volatile compound can be manipulated by treating the dried corn silk through certain combination of heating temperature and time. At different combinations of temperature and time, there are various types of amino acid and reducing sugar reaction embraced of degradation of many compounds in herbs or plants to form different volatile profiles.

Historically, corn silk has been used for over than hundred years ago as remedies to cure kidney related-illnesses by oldfolks especially in the East Asian region, Europe and other parts of the world. However in Malaysia, peoples are not using corn silk for specific purposes compared to Phillipine, China and European countries. In China and some European countries, corn silk is used as a therapeutics healing inflammation of the bladder and prostate, uneasiness of urinary tract system, bloating, diuretic and heart illness (Ebrahimzadeh *et al.*, 2008; Velazquez *et al.*,

2005). Corn silk has been shown to lessen the premenstrual syndrome like stomach cramp and to promote relaxation (Hasanudin *et al.,* 2012). In other countries like Brazil, Vietnam many researchers have claimed that corn silk exhibited significant effect of diuretic activity (Maksimovic *et al.,* 2004; Velazquez *et al.,* 2005; Ribeiro *et al.,* 1988). Nevertheless, there was also disagreement between them (Du Nat *et al.,* 1992; Al- Ali *et al.,* 2003).

Corn silk has been reported to contain polyphenolic compounds which could act as a herbal drug (Maksimovic *et al.* 2005). Presently people have tendency towards consuming natural products from plant-based ingredients. It is thought that plant-based ingredients are safer to treat diseases while avoiding possible adverse effect from generic or synthetic drug commonly formulated in some medicines of supplements.

1.1 Problem Statement

In 2010, Malaysia has produced about 47,602 metric tonnes of corn (Ministry of Agriculture Malaysia, 2010). However, due to higher demand and insufficient supply of corn, Malaysia has imported approximately 3,280 metric tonnes of corn. From our observation, based on calculation, it is estimated that more than 20,000 metric tonnes of fresh corn silk was discarded. Corn silk is discarded due to to lack of knowledge on the healing properties on renal diseases, urinary problems, diuresis and other application of corn silk.

On the other occasion, 3rd National Health and Morbidity Survey has reported about 4.8 millions of peoples are diagnosed with hypertension (MOH, 2008). Hypertension is a major risk factor for cardiovascular, cerebrovascular and renal diseases. Commonly, diuretics likewise furosemide, hydroclorothiazide, chlorothiazide and amiloride are used to treat hypertension. These diuretics are given in order to increase the excretion of urine and Na^+. Occasionally, it reduced the hypertensive effect. Therefore, plants that have diuretic property also can be used to decrease the hypertensive effect.

By fully utilizing this agricultural by-product, it is believed that many value added products will be benefited. Indeed, the usage of corn silk in cyno-industry and food industry will help small scale farmers to increase their socio-economic status. Furthermore, the utilization of corn silk may help in sustaining our environment by reducing the level of carbon cycle.

1.2 Objectives

The overall idea of this study is raised based on the following doubts;

1) What kind of phytochemical compounds are present in corn silk?
2) Are there any differences in volatile compounds developed during different combinations of preheating temperature and time on corn silk?
3) Does local corn silk exhibited diuretic activity like it was claimed by other foreign countries? If it does;
    i) What are the median effective dose (ED_{50}) value of CAE and CME?

ii) What kind of compound that might be responsible for the diuretic activity in corn silk?

Therefore, in relation to these queries, the objectives of the study were constructed as below;

1) To identify the presence of non-volatile phytochemical constituents in corn silk extracts
2) To identify the volatile compound profiles after preheating with different combinations of temperature and time.
3) To investigate the diuretic activity of local corn silk extracts
   i) To determine the pharmacological ED_{50} value of CAE and CME.
   ii) To find the potential compound that has association with diuretic property.

1.3 Importance of the study

Although the diuretic activity of cornsilk has been well documented, there is some disagreement between them. This disagreement was found between researches which might have been influenced by the origin and geographical of corn silk since there was such study indicating that there are many factors which influence the presence of compounds in plants. In addition, the local folk have never acknowledged the pharmacological activity or therapeutics usage of corn silk.

Therefore, the present study provides important scientific information related to the ethnomedicinal use of therapeutic properties of local corn silk.

Moreover, the present study originally is the first in endowing with a comprehensive finding on volatile profiles during different temperature and time as well as on preclinical evaluation of the diuretic activity. This effort also highlights the pharmacological diuretic effective dose of both aqueous and methanolic extracts of corn silk. These findings would add more value to both food and pharmaceutical industries.

CHAPTER 2

LITERATURE REVIEW

2.0 Medicinal Plant and Crop Production in Malaysia

About 14,500 species of blossoming plant are available in the Malaysian forest and approximately 1,200 of them are reported to have therapeutic properties (Handa *et al.,* 2006). Food and Agriculture Organization (2002) has reported that there are about 200 species of plants being used as the main ingredients for traditional remedies. Sometimes, historical circumstances and cultural beliefs leads to the practice of traditional medicine compared to modern medicinal practices. In the rural area, the aborigines are exclusively depends on local medicinal plant to treat certain diseases. In addition, the usage of herb or plant is an alternative approach to treat illnesses despite of end up using substitute drugs.

Most of the medicinal plants species are discovered wildly, while some of them are cultivated in the farm. Some of the regular remedies and aromatic plants are used as a major ingredient in local herbal product namely aloe vera, candletree, citrus, tumeric, cinnamon, *tongkat ali*, jasmine, ginger, black pepper and many more. These plants are used as the main ingredient in hair care and facial cleanser, antiseptic, cosmetic, perfumery-base, cleanser, food additive, spices, fragrant,

aphrodisiac, tonic base and as food flavour (Handa *et al.*, 2006). The list of commonly consumed herbs by people in the Southeast Asia countries including Malaysian is shown in Table 2.1.

Studies have been carried out by local research institutes and universities to investigate the biochemical and pharmacological aspects of plant compounds. These studies found that phytochemicals may lead to bioactivity which improved men's health. Meanwhile, the Ministry of Health Malaysia (2009) has reported that in January 2006, an establishment of the Traditional and Complementary unit has been approved by Malaysian Cabinet in selected hospitals including Kepala Batas Hospital in Penang, Sultan Ismail Hospital in Johor and Putrajaya Hospital in Federal Territory of Putrajaya. The realization of the initiated project is en route for assimilation of traditional and complementary medicine into public healthcare systems.

The Ministry of Agricultural Malaysia had endorsed the statistic of crop production in Malaysia during 2010 (Appendix I). This crop production consists of paddy, palm fruits and natural rubbers. Palm fruits is the main commodity contribute to sustain Malaysian economy with the production over 64,282,700 metric tonnes followed by paddy and natural rubbers. In addition, these major crops especially palm fruits and natural rubbers are also exported. While other crops with second-rate production mostly consumed domestically (Appendix II). Fruit productions were reported as the highest second rate production with 1,767,800 metric tonnes followed by vegetables, flowers, coconuts, short-term crops and others, with production below than 100,000 metric tonnes. This shot-term crop is known as a

Table 2.1

List of herbs commonly used in Southeast Asia

Scientific name	Local name	Medical use	Part used
Hibiscus sabdariffa L. [a,b]	Roselle	Urinary ailments	Petal
Allium cepa L. [c,f]	Onion	Sting	Bulb, juice
		Insomnia	Bulb
		Croup	Bulb, juice
		Build and clean blood	Bulb
		Prevent cold	Bulb, juice
Allium sativum L. [c,d,f]	Garlic	High blood pressure	Bulb
		Clean blood	Bulb
Brassica oleracea L. [c]	Cabbage	Upset stomach	Leaf
		Clean bowels	Leaf
		Expel worms	Leaf
Cinnamomum verum J. Presl. [c]	Cinnamon	Toothache	Bark
Citrullus lanatus [c]	Watermelon	Clean kidneys	Seed
Citrus limon L. [c]	Lemon	Cold and flu	Juice
Daucus carota L. [c]	Carrot	Improve eyesight	Root
Lectuca sativa L [c]	Lettuce	Insomnia	Leaf
Musa paradisiacal L. [c]	Banana	Leg cramps	Fruit
		Diarrhea	Fruit
Piper nigrum L. [c]		Earache	Seed
	Black pepper	Sore throat	Seed
Solanum lycopersicum L [c]	Tomato	Nausea and upset stomach	Fruit
		Hangover	Juice
Solanum tuberosum L [c]	Potato	Cold and flu	Tuber
		Indigestion and nausea	Tuber
		Wart	Tuber
Syzgium aromaticum L. [c]	Clove	Toothache	Flower bud
Vitis vinifera L. [c]	Grape	Build and clean blood	Fruit
		Arthritis	Fruit
		Cold and flu	grain
Orthosiphon stamineus[e]	Cat's whiskers	Diuretic	Leaf/aerial part
Zea mays L. [c]	Corn	Measles	Grain
		Rash	Grain
		Clean kidneys	Silk
Zingiber officinale Roscoe [c]	Ginger	Cold and flu	Rhizome
		Nausea and stomach ache	Rhizome
		Menstrual complaints; cramps, nausea and the late onset	Rhizome

Alphabets in first column a-Herrera *et al.* (2004), b- Wang *et al.* (2000), c- Cavender (2006), d-Pantoja *et al.* (2000), e- Arafat *et al.* (2008), f- Benkeblia (2004).

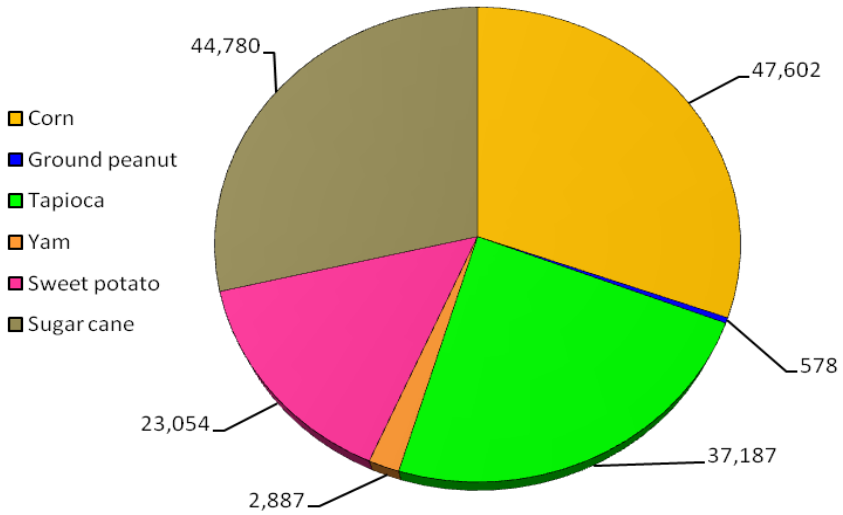

Figure 2.1 Production of short-term crop (metric tonnes) in Malaysia (MOA, 2010).

rotational crop planted during particular seasons. These crops are corn, ground, peanut, tapioca, yam, sweet potato and sugar cane (Figure 2.1).

Corn production is the highest among short-term crops and tagged along narrowly by sugar cane, tapioca and sweet potato. Apart from that, both yam and ground nut are insignificant harvested crop. According to United State Department of Agriculture (2013) database, the world agricultural production of corn during year 2010 was 831.36 million metric tonnes (Appendix III). The production was contributed mostly by the United States followed by China in the amount of 316.17 and 177.25 million metric tonnes, correspondingly. The rest of the production was contributed by other countries including four countries in the Southeast Asia for instance Indonesia (6.8 million metric tonnes), Philippines (7.27 million metric tonnes), Vietnam (4.65 million metric tonnes) and Thailand (4.20 million metric

tonnes). Although Malaysia was not listed in the world production of corn, it produced about 47,602 metric tonnes in 2010.

2.1 Natural Product as Common Therapeutic Agents in Treating Illnesses

Presently, there is an increase interest in exploring and finding information on health benefits of herb and botanicals by consumers. In parallel, there are also increased numbers of published articles claiming therapeutic effects on some plants and herbs. In folk medicine, herb was commonly used to treat many illnesses like hepatic problems, excessive menstruation, hypertension, urinary problems (Esiyok *et al.*, 2004), gastric ulcer (Tournier *et al.*, 1999) and fragile bone (Muehlbauer, 2002). Naturally, these herbs contained various active compounds which have a specific functionality. These plants and herbs act as diuretic, antimicrobial, antiinflammatory, antidiabetic, anticoagulant, antihypertensive, antispasmodic and antiflatulence.

Bitter gourd (*Mamordica carantia)* is one of the common plants used to treat diabetes. Singh *et al.* (2011) have claimed that bitter gourd juice reduced glucose level and also improved the structural abnormalities of peripheral nerves. In another study, Cummings *et al.* (2004) reported that bitter gourd improved the secretion of insulin. Furthermore, Umacigi *et al.* (2008) reported that oak galls *(Quercus infectoria)* extract has been used to decrease blood glucose and treat wound infection, as it posses antidiabetic and antiinflammatory activities. Mirghani *et al.* (2012) also reported the potential of lemongrass oil *(Cymbopogon citrates)* in

treating diabetes as it antidiabetic activity increased in line with increment of its concentration.

Garlic *(Allium sativum)* is popularly used as food ingredient and flavoring agent in most of culinary preparation. Besides that, their usage as traditional medicine to treat hypentension is well known (Pantoja *et al.*, 2000). Studies by Pantoja *et al.* (1996) and Ribeiro *et al.* (1988) have shown that garlic provide protection against platelet aggregation, atherosclerosis, strokes, coronary thrombosis, hyperglycemic and hyperlipidemic. Besides that, Ribeiro *et al.* (1988) also found that banana leaves and nutmeg have antihypentensive activity. Other than that, Talha *et al.* (2011) also reported on antihypertensive activity of tackweed *(Tribulus terrestris)*. Moreover, oak galls also have been used in treating lipid level and artheroscheloris plaque formation in rabbits (Gholamhoseinian *et al.*, 2012).

There are numerous plants associated with ethnomedical properties have been used in treating diuresis. Caraway *(Carum carvi)* fruits and tansy *(Tanacetum vulgare)* leaves have been reported to elevate urine excretion (Lahlou *et al.*, 2007). Arafat *et al.* (2008) reported on various activities of cat whisker *(Orthosiphon stamineus)* leaf and ariel part in diuretic activity, treating gout and eliminating kidney stone. In Thailand, root extracts of pineapples and papayas were used to treat urination problem (Sripanidkulchai *et al.*, 2001). The authors reported that there was significant increase in diuretic activity exhibited by these natural products. Tackweed *(Tribulus terreteris)* has been identified to show significant diuretic and contractile activity as well as to expel kidney stone (Al-Ali *et al.*, 2003). Other than that, tea leaves also claimed to have potent diuretic activity and could dissolved kidney stone

(Ratnasooriya *et al.*, 2004). Aissaoui *et al.* (2008) have reported that coriander seed has the ability to increase urine excretion. In addition, it was also used in treating indigestion, insomnia, flatulence, renal disorder and loss of appetite (El-Hilaly *et al.*, 2003).

2.2 History and General Description of Corn and Its Stigma (Silk)

The scientific name for the plant is *Zea mays* L. The name *Zea* was originated from Greek which means cereal or grain. The ephitet *mays* was thought to derive from the native Arawak word maiz or mahiz to describe the plant in America. The word was adopted by the Spanish crew of Columbus's first voyage who first collected the grain and brought it to Europe (Hyam and Phankhurst, 1995; Desjardin and McCarthy, 2004). Corn is a native plant in North America and widely grows around the world in medium to high climate countries.

Corn plant is one of the oldest cultivated crops in the world. It is annual grass, growing up to 4 m tall. Farham *et al.* (2003) reported that female inflorescences, the ears developed in leaf axil of the stalk which terminates the male florescences, the tassel. The leaves are arranged in two opposing rows along the stalk. Though the corn plants can growth up to 4 m height, their stems are not woody. To all appearances, corn plants resemble bamboo cane. It consists of leaves, stems, nodes and ears. Roughly, the leave is 5 – 10 cm wide and 50 – 100 cm long while the stems conventionally erect about 2 – 3 m with many nodes. Each nodes cast off flag-leaves. Normally, under the leaves which were close to the stem, the ears grow.

The ears are female inflorescences, tightly layered by soft husk and covered over by several layer of leaves (Figure 2.2). The ears hardly showed themselves until the emergence of the pale yellow silks at the end of the husks. The silk are elongated stigmas that resemble a bunch of hair. It is a green colour and then red or yellow at the edge of the stigmas. The thread of fresh stigmas is 10 to 20 cm length with light green or yellowish-brown in colour and diameter ranging from 654 - 627 µm (Wan Rosli *et al.*, 2010).

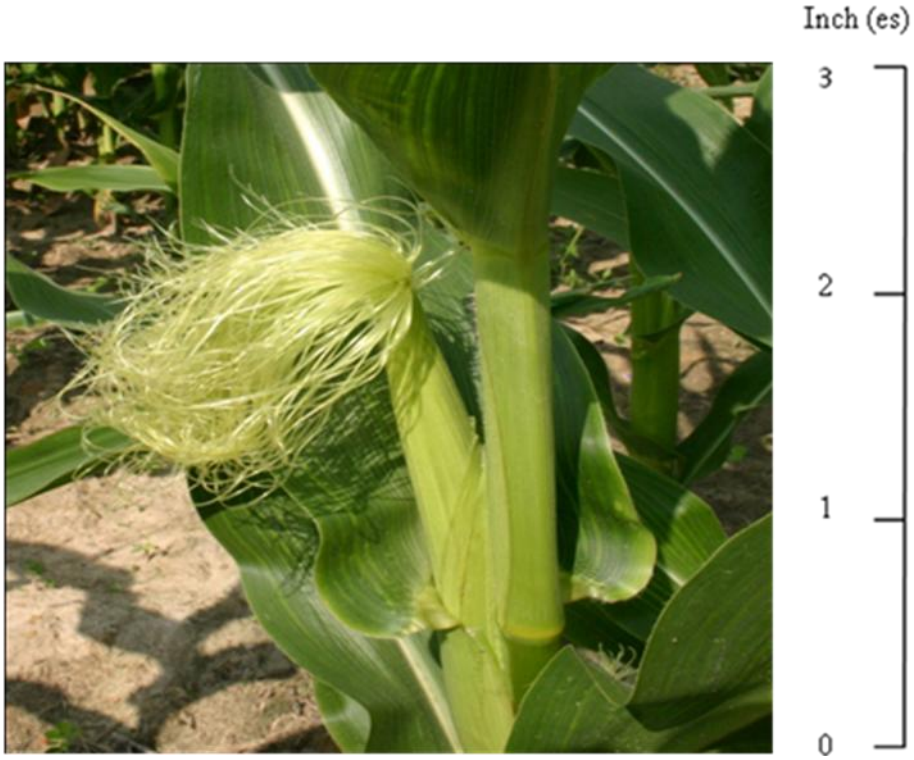

Figure 2.2 The emergence of corn silk in a corn farm located at Tendong, Pasir Mas, Kelantan. (Scale: 1 inch to 7 cm).

The stigmas are scientifically known as *Zea mays* hair. Alternatively, it is also known as corn silk, *maydis stigmata*, *Zea mays* stigmata, mother's hair and corn hair. Corn silk are known as different names in China, Indians and Malaysia likewise *yu mi xu, mother's hair* and *rambut jagung* accordingly. The corn silk name is

referring to the stigmas from the female flower of maize. Fresh maize has soft hairs which physically look like fine threads.

2.3 Taxonomy of the Corn Species

Zea mays species is a *Zea* genus and belongs to a tribe Andropogoceae. The subfamily is Panicoideae and family to Poaceae (USDA, 2005). There are 5 species included in the genus *zea* as shown in Table 2.2. Spesies of *Zea* that have been examined, mostly have chromosome of 2n = 20, except for the *Z. perennis* (perennial teosintes 2n = 40) (Ellneskog-Staam *et al.,* 2007). However *Zea mays* subspecies of *mays* is the only cultivated species. Other species and subspecies are wild grasses and are referred to teosintes.

They are many varieties of Z*ea mays*. Exotic varieties of corn are collected to add to genetic diversity when selectively breed for new local strains. Certain varieties of corn have been bred to produce many additional developed ears. The ears are called baby corn and particularly used as vegetables and are very popular among the Asean population. This baby corn is harvested about 40 days after planting. It is detached from the husk and the corn silk is also discarded. However, the husk is normally being processed for animal feed production.

2.4 Commercial Usage of Corn and Its By-products

Corn is one of the oldest grain being cultivated and among the most productive crop species with a global average yield of more than 4 tons per hectare

Table 2.2

The list of *Zea* Species and Subspecies

	Species	Chromosome Number	Subspecies	Synonym name
1	*Zea diploperennis*	2n = 20		
2	*Zea luxurians*	2n = 20		Euchlaena luxurians *Zea mays* ssp *luxurians*
3	*Zea mays* L.	2n = 20	*Zea mays* spp *huehuetenangensis*	
			Zea mays spp mays	Zea curagua Molina Zea indentata Sturtev. Zea indurate Sturtev. Zea japonica Van Houtte Zea mays cv alba Alef. Zea mays cv leucodon Alef. Zea mays var flavorubra *Zea mays* var *indentata* (Sturtev.) *Zea mays* var *indurate* (Sturtev.) *Zea mays* var *japonica* (Van Houtte) *Zea mays* var *saccharata* (Sturtev.) *Zea mays var tunicate* Larranaga Zea mays var vulgate Zea saccharate Sturtev.
			Zea mays spp *mexicana* (Schrad.)	Euchlaena mexicana Schrad. *Zea mexicana* (Schrad.)
			Zea mays spp *parviglumis*	Zea mays var parviglumis
4	*Zea nicaraguensis*			
5	*Zea perennis* (Hitch.)	2n = 40		Euchlaena perennis

Source: Department of Health and Ageing, Office of Gene Technology Rehulator, Australian Government (2005)

(Farham *et al.,* 2003). In 2009, Qi *et al.* (2012) has reported that global corn production (817 million metric tonnes) had exceeded wheat (682 million metric tonnes) and rice (678 million metric tonnes). In 2010, Malaysia has produced about 47,602 metric tonnes of corn (MOA, 2010). Due to lower supply and increased of demand, Malaysia has imported about 3280 metric tonnes of corn in 2013 (USDA, 2013). Corn can be directly consumed as food at various developmental stages from baby corn to mature grain.

Corn can be processed in a wide range of product both as an ingredient in foods and beverages. It is the major source of corn starch worldwide and being used as food ingredient either in its native form or chemically modified. Corn and cornmeal constitute a staple food in many region of the world. In Africa, corn meal is normally made into a thick porridge (Lin *et al.*, 2008). Corn meal can also be used as a replacement of wheat flour to make cornbread and other baked products (Martin *et al.*, 2012; Milanez *et al.*, 2006). Furthermore a processed corn namely corn grits, corn flakes (Martin *et al.*, 2012; Milanez *et al.*, 2006) and popcorn (Park and Maga, 2006) are popular snacks in most countries in the world.

Corns have multitude food, feed and industrial usage. The Department of Agriculture in the United State has reported that United State is the major trader of corn in the world markets, with roughly 20 % of them are exported to other countries. About 40 % of tropical areas and up to 85 % in developed countries which produced maize use corn as a stock feed (Farham *et al.,* 2003). The corn can be fed in form of green stock, dry forage, silage or grain. It is known as stover, which is the dried stalks and leaves of a crop after the grain has been harvested. Besides being

used as a food, corn usage is also applied in chemical and medicinal industries. Starch from corn can be made into plastics, fabrics, adhesive and many other chemical products. In addition, corn starch has been used as a thickening agent in some frozen food to maintain texture (Nebraska Corn Board, 2013).

Despite of that, the stigmas of the female flowers, known as corn silk are sold as herbal supplement. Corn silk is collected for medicinal purposes and can be used fresh or dried. In China, corn silk is commonly used as one of key ingredients in cosmetic products to give better affection such as to moisten the skin. In Mexico, corn silk is sold in bulk as a good food and safe medicine for renal problems in both adults and children. Some of the industrial application of corn silk is shown in Table 2.3.

Table 2.3
Industrial application of corn silk

Industries	Application	Bioactive properties/products
Biological	Microbial	Antifungal[a], antitumor [a], antimicrobial[b]
Pharmaceutical	Herbal drug Supplement	Antioxidant[c] , antidiabetic[d], antihypertension[e]
Cosmetical	Beauty product	Face powder[f]
Food and Beverage	Nutritional content Functional ingredient Flavour	Healthy drinks[e, g]

a-Kim *et al.* (2005); b-Nessa *et al.* (2012); c-El-Ghorab *et al.* (2007); d-Guo *et al.* (2009); e- Hasanudin *et al.* (2012); f-Revlon Consumer Products Corporation (2002); g-Cornsilk encyclopedia alternative medicine.

2.5 Acclaimed Therapeutic Effect of Corn Silk

Herb is a plant that is treasured for its flavour, scent, medicinal and other functional qualities. They have no persistent woody stem above the ground. Mostly, herb contains phytochemicals that provide significant effect to our bodies. In recent, majority of the world population depends strongly on plants for healing purposes and enhancement of their health status. Medical practices of natives American, Roman, Egyptian, Persian and Hebrew have reported that herbs were used comprehensively to treat nearly every known illness (Balch, 2006). Like other plant parts, corn silk also has a wide range of traditional usage because of their unique therapeutic properties. Other than healing activities, corn silk was consumed as tea, flavoring agent and food additive (Koedam, 1986; Yesilada and Ezer, 1989).

Corn silk have a possible potential usage as a traditional herb to treat diabetes and other diseases. It has diterpenes compound consists of carnosic acid and carnosol. These diterpenes function as activators of glitazones, which is used to treat diabetes (Rau *et al.,* 2006). Another study has recommended corn silk to be used as a hypoglycaemic food. This recommendation is due to the result of corn silk extract which was able to increase insulin level and heal wounded β-cell (Guo *et al.,* 2009). Therefore, corn silk was taught to be effective in controlling diabetes as shown by its capability to increase insulin level. Furthermore, Li and Yu (2009) have reported that flavonoid from corn silk was able to decrease human serum glucose level. It was also mentioned that superoxide dismutase activity was improved while malondialdehyde activity was decreased (Li and Yu, 2009). The authors suggested that flavonoid prevents oxidation activity by reducing the injury of β-cell. In addition, flavonoids

are probably resulting in the recovery of β-cell and enhancing the secretion of insulin to lower the blood glucose level (Li and Yu, 2009).

Previously, rhamnosyl-6-C-(4-ketofucosyl)-5,7,3′ ,4′ tetrahydroxyflavone (I) or also known as maysin has been successfully isolated from corn silk. This isolated flavone glycoside inhibited the growth of corn earworm (Waiss *et al.,* 1979; Snook *et al.,* 1995). Further study has shown that corn silk was opposed to insect attack (Guevera *et al.,* 2000). The flavones are expected to play an important role in preventing corn silk against insect threats and increasing the self-resistance of corn earworm larvae. Other than that, Ren *et al.* (2009) has identified 2 novel glycoside namely 2-O-α-L-rhamnosul-6-C-3-deoxyglucosyl-3-methoxyluteolin and 6,4-dihydroxy-3-methoxyflavone-7-O-glucoside in corn silk from China.

There are well described reports on the inhibition of antibody (IgE) formation due to the introduction of corn silk extract. A study has shown that IgE production was tremendously inhibited when the corn silk extract was given intranasally or intraperitoneally the day before primary immunization. In addition to that, IgE was also significantly reduced by the antigen in secondarily challenged of immunization (Namba *et al.,* 1993). Hence, the corn silk extract was suggested as clinically suitable to treat type 1 allergy diseases. Type 1 allergy is an immunological disorder related to hypersensitivity. It causes an excessive activation of white blood cell to produce IgE and result the inflammatory response at once. Kim *et al.* (2004) has also reported that the action of immune enhancement by non-starch polysaccharides in corn silk extract. Thus, the report have supported that corn silk have potential positive responses on immunological properties.

Recently another study has showed that corn silk has the ability as an antioxidant agent (Liu *et al.,* 2011; Eman, 2011; Ebrahimzadeh *et al.,* 2008; El-Ghorab *et al.,* 2007). Antioxidant plays an utmost role in protecting cells from the damage caused by unstable molecules known as free radicals. Free radicals are produced by oxidation reaction. Despite the ability to inhibit earworm growth, Liu *et al.* (2011) also found that flavones glycoside constituents have potent antioxidant activities in corn silk. These flavone glycoside constituents are isoorientin-2″-*O*-*α*-L-rhamnoside and 3′-methoxymaysin. Ironically, though the oxidation reactions are detrimental, it is somehow essential to life. Therefore, food or dietary supplement that contained antioxidant properties are important in food daily intake. Fruit and vegetables are good sources of antioxidants. It prevents our bodies from developing diseases like cancer, heart problem, stroke and Alzheimer (Di Matteo and Esposito, 2003). Other than that antioxidant properties can aid to rejuvenate our skin and also delay the aging process.

Suzuki *et al.* (2003) have reported the glycation inhibitory activity of corn silk. The authors identified two type of sugar biovinose in corn silk which was very rare and able to inhibit glycation similar to aminoguanidine. Besides that, Hu *et al.* (2010) and Hu and Deng (2011) reported that corn silk have an antifatigue activity. This activity is thought contributed by flavonoids compound in corn silk which shown affective mechanism in blood system. Swimming exercise was opted in the study in order to observe the biochemical changes in blood. Lactic acid in blood was inhibited and so the blood urea nitrogen was retarded. Subsequently the hepatic glycogen was increased, as well as exercise tolerance. Furthermore, Hu and Deng (2011) have showed that flavonoids from corn silk provided protection against

oxidative stress due to extensive exercise. As a result, lipid oxidation was impeded while antioxidant enzymes level was increased.

On the other hand, corn silk has been used to treat chronic nephritis, benign prostate hyperplasia, gout and cystitis (Ribeiro *et al.,* 1988; Maksimovic *et al.,* 2004; Maksimovic *et al.,* 2005; Tahraoui *et al.,* 2007). Corn silk has been suggested to assist in the treatment of renal disease and passing stone from the kidney and urinary tract (Maksimovic *et al.,* 2005; Velasquez *et al.,* 2005), lowering blood pressure and even lessen rheumatism symptoms. Corn silk was also claimed to exhibit potent antiprostatitis activity and antispasmodic properties (Buhner, 2007). In other aspect, Buhner (2007) reported that corn has a remarkable curative property in the treatment of kidney and bladder problems.

Besides that, corn silk was also used to treat infections and cystitis. It helped to reduce the frequent and painful urination, as well as the risk of prostate disorder (Steenkamp, 2003). Moreover, the use of corn silk has helped to encounter bed-wetting, bloating and edema due to its ability to remove excessive water from body tissues. Instead of treating urinary illnesses, it was said to have ability in lessening the effect of premenstrual syndrome and promoting relaxation (Hasanudin *et al.,* 2012).

2.6 Nutrient Composition of Corn Silk

Chemical composition of fresh corn silk contained of 1.2 % of fat, 0.18 % of protein and 7.6 % of ash (Wan Rosli *et al.*, 2008). On the other part, dried corn

silk contained about 12.96 % of protein, 1.27 % of fat, 5.28 % of ash and 48.5 % of total dietary fiber (Nurhanan and Wan Rosli, 2013). The result obviously was low for daily dietary intakes hence it was comparable to other studies reported on dried leaves. In comparison to other dried leaves, Hussain *et al.* (2009) have reported nutritional composition of *Corriandrum sativum, Ammonum sulbulatum* and *Punica garanthum*. The protein content of the leaves was lower (11.75, 5.44 and 2.84 %, respectively) but higher in fat (9.83, 2.08 and 4.91 %, respectively) and ash (8.03, 6.97 and 5.75 %, respectively).

Many essential minerals and vitamins are listed as micronutrient class hence only a trace of amount is required by our body. The deficiency and excessive amount of micronutrients in body may lead to certain illnesses. Thus, an adequate amount of mineral and vitamin for daily consumption has been recommended in Dietary Reference Intake was illustrated in Appendix IV and V, respectively. Wan Rosli *et al.* (2010) have reported that corn silk contained some essential minerals such as potassium, iron, silica and vitamin B and K.

2.7 Non-volatile Bioactive Compounds in Corn Silk

Like other plants and herbs, corn silk also contained various phytochemicals. According to El-Ghorab *et al*, (2007), corn silk contained proteins, vitamins, alkaloids, tannins and mineral salts (Namba *et al.,* 1993). Other than that, it consists of flavanoids (Maksimovic and Kovacevic, 2003), carbohydrates (Tang *et al.,* 1995) and steroids (Abdel-Waheb *et al.,* 2002). Corn silk also contained several bioactive compounds like antocyanins, p-coumaric acid, vanillic acid, protocatechuic

acid, derivatives of hesperidin and quercetin, p-coumaric and ferulic acid (Ebrahimzadeh *et al.*, 2008).

Most of bioactive compound reported by Ebrahimzadeh *et al.* (2008) were classified in family of flavonoids. These flavonoids are important in reducing total cholesterol (Warshafsky *et al.*, 1993; Matsuura, 2001), platelet aggregation (Tzeng *et al.*, 1991), eicosanoid synthesis and inhibiting tumor initiation or promotion (Yang *et al.*, 2000). Most flavonoids compound can be found in grapes, soybeans, legume, tomato, garlic, broccoli, lettuce, chocolate, green and black tea and herbs.

Anthocyanins, another compound of flavonoids has visible pigment color of purple to red. This compound possessed antioxidant property to scavenge free radicals in the body. It also has an ability to retard tumor formation by interfering with the carcinogenesis process (Hou, 2003; Kang *et al.*, 2003). Besides that, anthocyanins were able to improve night vision (Nakaishi *et al.*, 2000). The benefit of anthocyanins in plant has been conventionally practiced in old folk medicine. Instead of corn silk, anthocyanins can also be found in *Hibiscus sp* (Wang *et al.*, 2000), bilberries (Mazza and Miniati, 1993) and blueberries (Smith *et al.*, 2000). Anthocyanins has been reported to function as a functional food which helps in the prevention of obesity and diabetes. It impedes the increment of body weight and adipose tissue (Tsuda *et al.*, 2003).