Aqueous, Alcoholic Treated and Proximate Analysis of *Maydis stigma (Zea mays* Hairs)

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ABSTRACT

Globally medicinal plants and herbs have long been commercially exploited and referred as traditional medicines of tangible value. This medicinal plant and herb is closely associated with local complementary alternative medicine. Sadly, many of this traditional natural available product of local plants are poorly elucidated or scientifically proven. With the advent of sophisticated techniques and new approach in high-resolution microscopy, this medicinal plant prowess can be revisited and harness. Of interest in this paper, is a local plant that was once quoted in the American National formulary for prostate problems. The longitudinal surface texture of the corn silk thread fibre was lamellated and non porous when intact protuberances were present. The silk thread sample was recorded the diameter ranging from 654 – 627 µm. When cut/section, the microtubules are present and gives the features of a thru and thru opening while some of them seems occluded. The surface of the thread looks smooth and clean of artifacts or microorganisms suggestive that ethanol acts a viable chemical fixative to corn silk presentation. Ethanol extracts contained 0.41% nitrogen compounds while water extract were at the richest source (1.40%) followed by fresh corn silk (0.18%).

KEYWORDS: Corn silk, Scanning Electron Microscope (SEM), proximate analysis, AAS

INTRODUCTION

Local medicinal plants and wild herbs are of great significance to the sustainable health of individuals and its geo-communities. People on all continents and ethnics have long applied poultices and imbibed hundred of infusions, if not thousands, indigenous plants, dating back to prehistory time. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most of these are secondary metabolites, of which at least 12,000 have so far been scientifically isolated, a number estimated to represent less than 10% of its total metabolites (Cowan 1999). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. While some, such as terpenoids, give plants their odors; and others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor attributes (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices are used by humans to season food that yield useful medicinal compounds (Cowan 1999).

Presently, the use of traditional medicines remains widespread in developing countries while the use of complementary alternative medicine (CAM) is increasing rapidly in developed countries. Prostate cancer patients and those with benign prostatic hyperplasia (BPH) are increasingly exploring the potential use of CAM especially when compared with the risk of mortality and long-term morbidity associated with interventive clinical surgical procedures (Steenkamp 2003). Herbals which hold potential promise are mentioned, although much elucidation and tangible research is still found wanting. Medicinal plants and herbs are of great importance to the health of individuals and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species have been studied for their application (Kochhar *et al*. 2006).

Corn silk (*Zea mays* L.) refers to the stigmas of the maize female flowers. Historically, its have been used as a therapeutic remedy for various ailments such as the inflammation of the urinary bladder and prostate as well as treatment for irritation within the urinary system. To date, numerous commercially viable products prepared from corn silk are available (El-Ghorab *et al.* 2007). Although not scientifically proven, rhetorically, corn silks have long been reported in ancient literatures to be able to assists with prostate problems, bed-wetting, carpel tunnel syndrome, edema and obesity. It has also been used to lessen the effects of premenstrual syndrome, and said to promote relaxation. Corn silk was also reported to be useful to treat urinary infections and cystitis. It is helpful for frequent urination caused by irritation of the bladder and urethral walls as well as for difficulty in passing urine, e.g. prostate disorders. It soothes and relaxes the lining of the urinary tubules and bladder, thus relieving irritation and improving urine excretion (Steenkamp 2003).

The biological activities of corn silk constituents are well cited in literatures. These includes antibiotic activity toward corn earworm by a flavone glycoside maysin (Maksimovic and Kovacevic 2003), attractant activity toward corn earworm (Guevara *et al*. 2000), inhibition of IgE formation by glycoproteins (Tsuneo *et al.* 1993), immune enhancement by nonstarch polysaccharides (Guevara *et al.* 2000), anticoagulant activity by neutrosugar/aminosugar derivatives, phytochemical and biological study (Abdel-Wahab *et al.* 2002), purification and characterization of an anticoagulant from corn silk (Sang-Kyu and Hye-Seon 2004), phytomedicines as a treatment of benign prostatic hyperplasia (Steenkamp 2003), glomerular function and potassium urinary excretion (Velazquez *et al.* 2005) and volatiles inhibit cultures of *Aspergillus flavus* (Zeringue 2000). Other than these reported biological activities, some local species are used as tea, and they were powdered as food additive and flavorings agents in several regions of the world (Koedam 1986; Yesilada and Ezer 1989).

There are indications that utilization and acceptance tendency towards medicinal herbs to give relief and treat human ailments are globally very positive although there are side effects. The interest toward elucidating the chemical composition of medicinal herb products is also growing as commercializing exploitation increases (Basgel and Erdemoglu 2006).

Various information on the *Zea mays* hairs are cited in the literatures but sadly; there are lacunae especially in its ultra structural study using high-resolution electron microscopy. Thus attempt is now being made to utilize these sophisticated high resolution technology in an attempt to develop corn silks as viable natural products post structural, chemical and elemental characterization. The aim of the study is thus to elucidate the ultra structural elucidation and determining proximal composition of aqueous and ethanolic extracts of local *Zea mays* hairs.

MATERIALS AND METHODS

Sample preparation

Fresh hairs of *Zea mays* L (dried cut stigmata of *Zea mays* L, Poaceae female flowers) were harvested from Pantai Cahaya Bulan, a coastal district area of Kota Bharu, in the state of Kelantan, Malaysia. 500g of fresh plant was collected, cleaned and gently washed under running tap water. Excessive water was drained off before the sample was divided into 2 groups for research interest. One half of the corn silk thread samples were subjected to morphological characterization while the other half of the samples were used for analyse with water and ethanolic extraction.

Scanning Electron Microscopy

Ten grams of the freshly harvested cornsilk samples were either (a) directly soaked in 2.5% glutaraldehyde and then rinsed with phosphate buffer solution or (b) soaked and agitated

(Rotamax 120, Heidolph, German) for 2 hours in industrial ethanol. The samples were then freeze dried (IIshin model TFD5505 Korea) for 24 hours. The dried samples were coated with a thin-layer of gold in a vacuum evaporator (Baltex SCD005 Sputter Coater, Hi-Tech Germany) and their structure and morphology were studied in a LEO 1455 VPSEM under 5.5 Pascal pressure, using the secondary electron mode, at a working distance of 10 mm at 5.0 kV (Wan Rosli *et al*. 2007a).

Aqueous Extraction

500g of freshly harvested samples were cut into small pieces, soaked in distilled water for 10 min [in the ratio of sample to water 1:5] and homogenized using a Waring blender for 2 min. The extract was filtered using filter paper (Whatman no. 1) and the filtrate was collected. The remaining residue was re-extracted twice and then the two extracts were combined. The water was removed by rotary evaporator (Laborota 4000, Heidolph German)) at 50^{o}C to obtain semi dry extract. The aqueous extract was then freeze-dried for 24 hours. Dried extracts were placed in a bottle, stopped and then stored at -20^{o}C until used.

Ethanolic extraction

For this extraction, 100g of the samples soaked in 500 ml 97% ethanol (AR grade) and shaked using a shaker (Rotamax 120, Heidolph German) for 24 hours. The extract was filtered using Whatman no. 1 filter paper and then collected. The remaining residue was re-extracted twice, and then the two extracts were combined. The ethanol was removed by rotary evaporator (Laborota 4000, Heidolph German) at 50^{o}C to obtain dry extract. The ethanolic extract was produced in triplicate. Dried extracts were placed in a bottle, stopped and then stored at 4^{o}C until used.

Proximate analyses

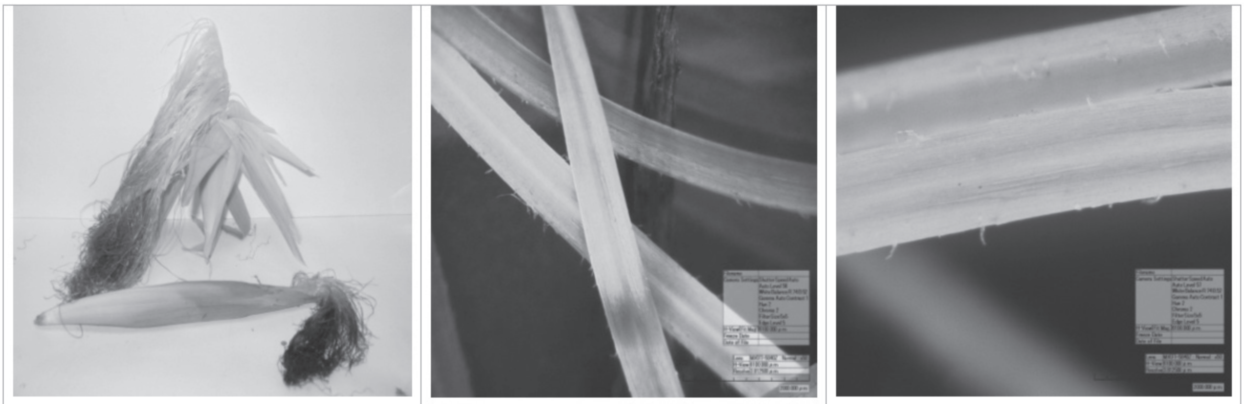
Proximate analyses were conducted using AOAC (1995) for moisture, ash, soluble dietary fibre (SDF), insoluble dietary fibre (ISF), total dietary fiber (TDF), crude protein by nitrogen conversion factor of 6.25 and crude fat content using the semi-continuous extraction [Soxhlet] method.

RESULTS AND DISCUSSION

The corn silk general morphology, when observed fresh, is flat fine silk-like threads. The thread of fresh corn silk was length from 10 to 20 cm long and of a light green or yellowish-brown in colour (Figure 1). The protuberances or granular hairs were observed out pouching from the surface of the corn silk threads. Corn silk consists of various chemicals, including proteins, vitamins, alkaloids, tannins and mineral salts, carbohydrates, steroids, and flavonoids as well as volatile chemicals (Kwag *et al.* 1999).

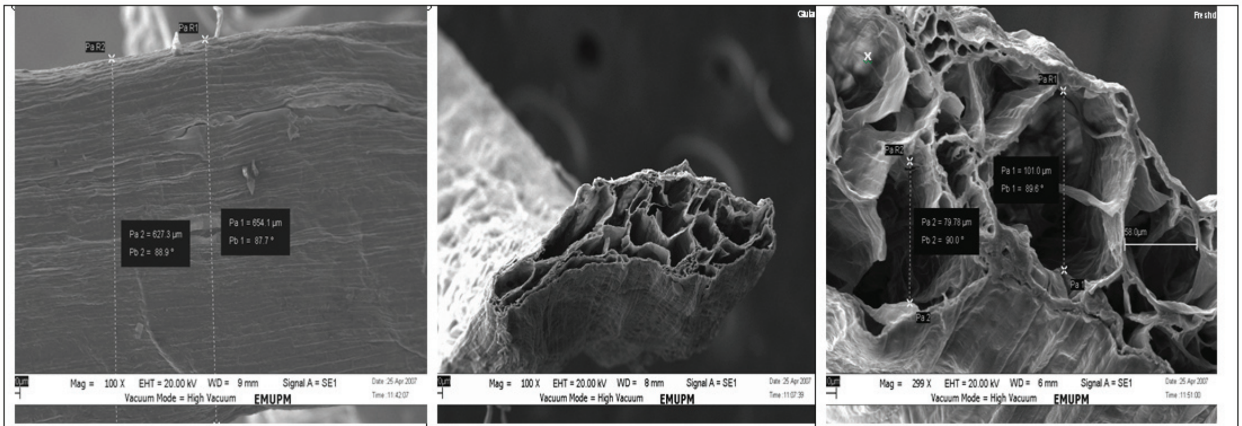
Figure 2 shows scanning electron photomicrographs of fresh dried corn silk. The surface texture of the corn silk thread fibre was well arranged, homogenous and intact. This fresh corn silk thread sample was recorded the diameter ranging from 654 – 627 μm. Cross sectional of fresh corn silk sample shows some microtubule with various size (2b). Some of the microtubules can be seen thru and thru observation and some of them were occluded. The diameter of the porous tubules was ranging from 58 – 101μm. The putative pebble globule-like structure in one of the enclosed microtubule (x) could represent a chlorophyll bud.

Figure 3 elucidates electron photomicrographs morphology of the corn silks treated with ethanol. Each thread is made up of homogenous incremental folds. These folds are actually the outermost wall of numerous microtubules observed under cross-sectional cut presented within the belly of each thread. However the diameter and circumference of the microtubules are irregular and non-homogenous. At low magnification, the width of the thread was observed in the range between 317 to 340μm (3a). Prominent outpouching protuberances were obviously observed on the threads surface. They protrude for every 200 – 300 μm intervals along the longitudinal extension of the



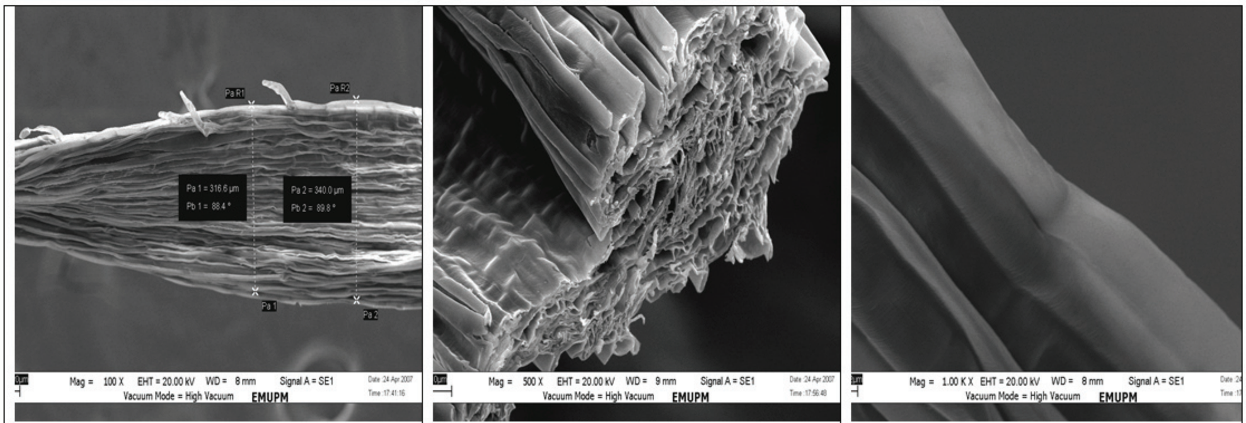
1a | 1b | 1c

Figure 1: Digital image (1a) and HIROX KH7700 photomicrography (1b and 1c) of the fresh harvested non-treated corn silk samples



2a | 2b | 2c

Figure 2. VPSEM Photomicrograph images of corn silk samples treated with ethanol observed at low magnification (a) cross section view (b) and higher magnification (c) to reveal the microtubules.



3a	3b. This image won the best photomicrograph on the micrograph competition of the 6th ASEAN Microscopy Conference 2007 held in Impiana Cherating Hotel, Pahang, Malaysia 10-12 Dis 2007	3c
Lamellated, stacking feature		**Smooth, polish surface at the outer surface**

Figure 3: VPSEM Photomicrograph images of corn silk samples treated with ethanol viewed at 100X (3a), 500X (3b) and 1000X (3c) magnification

hairs. Each one of them emanates from a single surface cell to increase the area through which water can be absorbed into the whole corn silk.

The overall topographical surface of the ethanol treated thread looks smooth and clean of artifacts or microorganisms. This presentation is highly suggestive that ethanol if any, causes minimal lipid degradation, thus acts functionally a good fixative to the said tissue thus the smooth non-denatured appearance. The cross sectional corn silk thread photomicrograph of lamellated and slightly flattened 'collapsed' microtubules presented at the cut terminal end of a *Zea mays* hair (3c). Although, there was an efforts to explore individual microtubule of the sample preparation, it seems that the whole tubule was well-defined and have totally collapsed and occluded. The diameter of these microtubules were varies from 58-100 µm

One of the main structural polymers of plant cell walls is known as cellulose. Many parallel cellulose chains are held together by hydrogen bonds between hydroxyl groups of the glucose monomers to form a cellulose fibril with indefinite length embedded in a gel matrix composed of hemicelluloses, lignin and other carbohydrate polymers (Yu *et al*. 2005). Phloem carries organic substances made within the plant, while the inner part, the xylem, transports water and salts up the plant from its roots origin (Cowhig 1974).

The proximate composition of different extract samples are presented in Table 1. Moisture content of fresh corn silk plants accounted for 83.91% while other extracts recorded less than 1%. Ethanol extracts contained minimum nitrogenous compounds (0.41%) while water extract were richest source of it (1.40%) followed by fresh corn silk (0.18%). There was significant differences ($P<0.05$) in nitrogenous compounds content of these medicinal plants. There are a very large number of different nitrogen containing substances exist in plants. Nitrogen is first available to the plant in the form of ammonia, produced either from nitrogen fixation in the root or from enzymatic reduction of absorbed nitrate in shoot and leaf. Nitrogen first appears in organic form as amino acids, which are involved in the biosynthesis of practically all the other nitrogenous plant compounds from the proteins to the alkaloids, amines, cyanogenic glycosides, purines, pyrimidines and cytokinins (Harborne 1998).

Table 1: Proximate value, dietary fibre and caloric value of water and ethanolic extracts of the corn silks

Sample	Water Content (%)	Crude Lipid (%)	Nitrogenous Compounds (%)	Ash (%)	SDF (%)	IDF (%)	Total DF (%)
Fresh cornsilk	83.91 + 0.37 ^{a}	1.28 + 0.06 ^{b}	0.18 ± 0.01 ^{c}	7.60 ± 0.10 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00^{b}	0.0 ± 0.00^{c}
Aqueous extract	< 1.00 ^{b}	0.17 ± 0.08 ^{c}	1.40 ± 0.21 ^{a}	21.55 ± 0.20 ^{a}	0.05 ± 0.01 ^{a}	0.00 ± 0.00 ^{b}	0.05± 0.01^{b}
Ethanol extract	< 1.00 ^{b}	28.63 ± 0.96 ^{a}	0.41 ± 0.11 ^{b}	6.11 ± 0.13 ^{c}	0.00 ± 0.00 ^{b}	0.08 ± 0.01 ^{a}	0.08 ± 0.01^{a}

$^{a-c}$ Mean values within the same column bearing different superscripts differ significantly ($P<0.05$)

Crude lipids content of ethanolic corn silk extract recorded the highest value (28.0%) compared to fresh (1.30%) and water extracts (0.20%). The lipids in plant leaves are important as membrane constituents in the chloroplasts and mitochondria (Harborne 1998). The high amount of crude lipids detected in this ethanolic extract of corn silk may be due to the fact that the lipids are more extractable with alcohol (Harborne 1998). Petroleum ether has been used comprehensively to extract lipid in various samples but its also able to dissolve other components such as steroid, carotenoids, vitamin E and chlorophyll (Cowan 1999). Based on this present study, a lot more work need to be conducted to explore the existence of various type of carotenoids which is pro-vitamin A and vitamin E homologs in the aqueous and ethanolic extract of corn silks.

Water extract sample recorded the highest amount of ash (21.55%) compared to the fresh corn silk and ethanolic extract which recorded lower percentage of ash (7.60 and 6.11 %), respectively. The ash of a foodstuff is the inorganic residue remaining after the organic matter has been burnt away. Further analysis of macro and micro-minerals using atomic absorption spectrophotometer (AAS) of the ash revealed that Ca, Mg, Fe, Na, K, Cu, Zn and Mn present at the highest concentration in water extract corn silk as compared to other samples ((Wan Rosli *et al*. 2007b).

Dietary fibre plays an important role in human health. Numerous studies have shown that vegetarian or individual with high fibre intakes have blood pressures measure very lower than those with low fibre intake. Dietary fibre consists mainly of cellulose, hemicellulose and lignin, which exert different physiological effects on human health (Zia-ur-Rehman *et al.* 2003). In the present study, the low soluble dietary fibre (0.05 %) was detected only in water extract samples while the insoluble dietary fibre was only detected in ethanolic extract of corn silks. However the dietary fibre was not detected in fresh corn silk. These data suggestive that the corn silks is not a good source of dietary fibre.

CONCLUSION

This research pilots the therapeutic possibilities of the marketable local *Zea mays* hairs. More works need to be conducted to elucidate its viable potentials as a prelude to a tangible therapeutic entity. Further investigations on its anti-oxidative properties especially on the existence of individual antioxidant compounds and anti-microbial properties will now be pursued. Animal feeding experiment to observe initially, clinical prostatic ailments, correlated with *Zea mays* hairs will also be conducted. The nutritional values investigating the existence of carotenoids (pro-vitamin A), vitamin E and amino acid profile will also be studied.

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