

Phytochemicals screening and total phenolic content of Malaysian *Zea mays* hair extracts

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Abstract

In the present study, Malaysian *Zea mays* hair extracts are screened for the occurrence of bioactive compounds. The results positively showed the present of flavonoids, saponin, tannins, phlobatannins, phenols, alkaloids and cardiac glycosides in both aqueous and methanolic extract of *Zea mays* hair. Terpenoid compounds however present only in the methanolic extract sample. In addition, the total phenolic content (TPC) in aqueous extract was significantly higher (42.71 ± 0.87 $\mu\text{g/g}$ of tannic acid equivalent (TAE)) compared to methanolic extract (40.38 ± 1.10 $\mu\text{g/g}$ of TAE). The findings suggested that phytochemicals present in *Zea mays* hair are potentially beneficial as therapeutic and antioxidative agents in pharmaceuticals, food and other related industries.

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Introduction

Traditional obsess in folk medicinal plants is well known since thousand years ago. Commonly the ailment incidence in the rural area is treated with local plants that contain many pharmaceutical constituents (Sofowora, 1982). Due to the effectiveness in treating various ailments, *Zea mays* hair is frequently chosen and worldwidely used as an old folk therapeutic agent. *Zea mays* belongs to family Gramineae (Canadian Food Inspection Agency, 1994). It can be found in tropical regions for instance North American, China, India (U.S Grain Council, 2010) and various parts of the world including Malaysia. Though, *Zea mays* crop production in Malaysia is not significant. *Zea mays* hair is found inside the husks of corn. It hardly shows themselves until the emergence of the pale yellow silks from the end of the husks. The silk are elongated stigmas that resemble bunch of hair.

Zea mays hair contains various bioactive constituents comprise of protein, vitamin, minerals and salts (Namba *et al.*, 1993), flavonoids (Maksimovic and Kovacevic, 2003), steroid (Abdel-Waheb *et al.*, 2002), carbohydrate (Tang *et al.*, 1995) and volatile components (Zeringue, 2000). Phytochemicals present showed potential activities against hypoglycaemic (Guo *et al.*, 2009). On the other aspect, *Zea mays* hair extract has been reported to increase insulin level and

healed injured β -cell. *Zea mays* hair also has been claimed to have immunology activity. It is said to treat hypersensitivity related to type I allergy disease (Namba *et al.*, 1993; Kim *et al.*, 2004). Besides that, *Zea mays* hair has been documented to exhibit anti-proliferative effect on cancer cell line (Habtemariam, 1998).

Zea mays hair has been claimed to have effect more particularly on renal diseases including chronic nephritis, benign prostate hyperplasia, gout and cystitis (Ribeiro *et al.*, 1988; Maksimovic *et al.*, 2004; Tahraoui *et al.*, 2007). It helps to pass stone from kidney and urinary tract and prevent the inflammatory effect. Besides, *Zea mays* hair has anti-prostatitis and anti-spasmodic activities (Buhner, 2007). Recently, *Zea mays* hair has been reported to have anti-fatigue activity. The flavonoids compound in the hair has affected the mechanism in the blood. Hence it increased the hepatic glycogen and consequently, increased the exercise tolerance (Hu and Deng, 2011). The hair has antioxidative properties. They protect cells from damages due to oxidation process in the body triggered by free radicals (Eman, 2011).

Phenols are naturally occurring compound in plants. Plant phenols are groups of antioxidant that inhibit various stages of cancer process (Wattenberg, 1992). Pharmacologically, phenols give protection against cardiovascular disease. On the other property,

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phenol plants can protect against lipoprotein oxidation (Hollman, 2001). Flavonoids are a group of phenolic compound. It can be found in fruits, vegetables, grains, barks, tea, flowers, root and wine (Middleton, 1998). It is well known to exhibit wide range of biological effects including anti-inflammatory, antibacterial, antiviral, anti-allergic (Hanasaki *et al.*, 1994), antioestoporotic (Hegarty *et al.*, 2000) and posses antioxidative and antitumor activities (Stefani *et al.*, 1999).

Tannins are water soluble polyphenols and principally found in edible plants. It is commonly refer as tannic acid. Tannins are anti nutritional compound. It is said to have antimicrobial activity (Schalbert, 1991). Phlobatannins is a fraction of tannins which was said to be as a diuretic property of the plant (Awoyinka *et al.*, 2007). This result suggests the possible claimed of *Zea mays* hair as a diuretic agent. There are many compounds of alkaloids. Alkaloids can be found mostly in fungi and plants. Alkaloids are well known as a toxic substance. However, they often posses pharmacological effects and are used as medications.

Terpenoids are the largest group of natural products. These lipids biologically produce steroid and sterols in animal body. Terpenoids can inhibit the *Candida albicans* growth (Zore *et al.*, 2011; Houghton *et al.*, 2003). Furthermore, it has anti inflammatory and antioxidant activities (Houghton *et al.*, 2003). Saponin can be classified as steroidal saponin or triterpenoid saponin or steroidal alkaloids. They showed such as persistent foam in aqueous solution when shaken vigorously. It is believed to form the mains constituents of many plant drugs and folk medicines. Hence, it is considered for numerous pharmacological properties (Estrada *et al.*, 2000). Saponin has been reported to have anti viral activity, antitumor, antifungal, anti parasitic and antibacterial capacities (Sparg *et al.*, 2004).

Cardiac glycosides shared a common structure consisting of steroid ring. There are one to four sugars attached to 3 β -OH group of cardiac glycosides. The pharmaceuticals action of cardiac steroid on heart is well known (Winnicka *et al.*, 2006). Moreover, the curative effect of cardiac glycosides on breast cancer already has been identified since 1979 (Winnicka *et al.*, 2006). Many previous studies related with *Zea mays* hair were concentrated only on the ability of this herb in different pharmacological aspects. However, the basic information on the types of phytochemical groups presented in this herb is scanty. Thus, the aim of this study is to investigate the presence of phytochemical constituents of Malaysian *Zea mays* hair and the yield of extracts.

Materials and Methods

Materials

Corn was purchased from Pasar Siti Khadijah in Kota Bharu. The variety used was vegetable corn. It was harvested during aged 45-50 days. The hair generally emerged 5-7 days before harvested. *Zea mays* hair was detached from the cobs and the inside tassel was collected. *Zea mays* hair was dried at 55 °C in the oven to achieve 10–11 % (w/w) of moisture content. Domestic blender (National; MX-895) is used to grind dried *Zea mays* hair into powder form. *Zea mays* hair powder with 60 μ m mesh size was used.

Preparation of aqueous and methanolic extract

Aqueous extract was prepared according to Sripanidkulchai *et al.* (2000), with slight changes by boiling 80 g of *Zea mays* hair powder with distilled water for 30 min. The ratio used was 1:15 (w/v). The solution was then filtered through filter paper (Advantec; No. 1) attached to the vacuum pump (Welch; 2545C-02) at 30-40 kPa. The filtrate was then heated on hot plate with temperature below 60 °C until 24 h and the weight was recorded. Methanolic extract was prepared by ordinary Soxhlet apparatus. Sixty grams of *Zea mays* hair powder was used, with a ratio of 1:4 (w/v) to methanol. Methanol is then completely removed using vacuum evaporator (Heidolph; Laborota 4000). The weight was recorded.

Phytochemicals screening

Aqueous and methanolic extract of *Zea mays* hair are subjected to preliminary screening of phytochemical constituents. The procedures were described by Sofowara (1993) and Harborne (1973). All the extract used was diluted to distilled water with a ratio 1:100 (w/v) except for the cardiac glycoside test.

Test for phenols

Two (2) ml extract were taken into water and warmed at 45-50 °C. Then 2 ml of 3% FeCl₃ was added. Formation of green or blue colour will indicate the presence of phenols.

Test for flavonoids (I)

One (1) ml extract was added to 1 ml of 10% lead acetate. It was gently shaken. A muddy brownish precipitate indicates the presence of flavonoids.

Test for flavonoids (II)

One (1) ml extract was added to 10% FeCl₃. The

mixture was shaken. A wooly brownish precipitate will indicate the presence of flavonoids.

Test for tannins

One (1) ml was added to 1 ml of 3% FeCl₃. A greenish black precipitate signifies the presence of tannins.

Test for phlobatannins

One (1) ml extract was boiled with 2 ml of 1% hydrochloric acid. The red precipitate signifies the presence of phlobatannins.

Test for alkaloids

One (1) ml *Zea mays* hair extract was stirred with 5 ml (1%) hydrochloric acid on a steam bath (60 °C) for 15 min and filtered. Test for alkaloids I: One (1) ml of Dragendorff reagent was added to 1 ml filtrate. The formation of cloudy orange was formed. Test for alkaloids II: One (1) ml of Mayer reagent was added to 1 ml filtrate. A slight yellow colour was appeared. Test for alkaloids III: One (1) ml of Wagner reagent was added to 1 ml filtrate. The observation of turbid brown colour indicated the presence of alkaloids.

Test for terpenoids

Five (5) ml extract was mixed in 2 ml chloroform. Then 3 ml concentrated sulphuric acid is carefully added to observe a reddish brown coloration between upper and lower layer.

Test for saponins

Approximately 0.2 ml extract was mixed with 5 ml distilled water. It was shaken vigorously for 5 min. Persistence of foams was the indicator for saponins.

Test for sterols (Salkowski's test)

Two (2) ml of concentrated H₂SO₄ was added to 2 ml of *Zea mays* extract. A red precipitate indicated steroidal ring.

Test protein-xanthoprotein

A few drops of nitric acid were added by the side of the test tube containing one (1) ml of *Zea mays* hair extract. A yellow colour is formed to indicate the presence of protein-xanthoprotein.

Test cardiac-glycosides

One hundred (100) mg extract is dissolved in 1 ml glacial acetic acid containing 1 drop of 3% FeCl₃. Then it is under layered with 1 ml concentrated sulphuric acid. The formation of brown ring at the interface indicates the presence of de-oxy sugar characteristic of cardenolides.

Total Phenolic Content (TPC)

The determination of TPC was conducted according to the methods described by Mohd Ilham *et al.* (2008). The aqueous and methanolic extract (100 mg) were weighed separately and dissolved in 1 ml of 1% hydrochloric acid in methanol solvent (v/v). The extracts were then centrifuged at 6000 rpm for 60 mins (Hettich Zentrifugen; Universal 32R). One hundred (100) µl of each supernatant were pipetted into a bottle after which 750 µl of Folin-Ciocalteu reagent (10 x dilutions) was added. The solutions were left to stand at room temperature (25 °C) for 5 minutes. After that, 750 µl of sodium bicarbonate (60 mg/ml) was mixed into the solutions and left to react in the dark for 90 minutes. Distilled water was used as a blank in the analysis. The absorbance of the samples was read at 725 nm by using UV-VIS spectrophotometer (Varians, USA). The TPC was calculated by comparing the absorbance with the tannic acid calibration curve according to the formula:

$$TPC (\mu\text{g/g}) = C \times V / g$$

where;

C = concentration of the tannic acid equivalent from standard curve (µg/ml)

V = volume of the extract used (ml)

g = weight of extract (g)

The contents were expressed as tannic acid equivalent (µg TAE/g).

Results and Discussion

A concentrated of both aqueous and methanolic extracts of *Zea mays* hair yield were 40.8% and 62.3%, respectively (w/w). The result shows that the yield of methanolic extract was higher than aqueous extract. It may be due to the different polarity of the solvent used. Table 1 showed the phytochemicals present in both extracts. Phenols, flavonoids, tannins, phlobatannins, alkaloids, saponins and cardiac glycosides were present in both aqueous and methanolic extracts. Whereas, terpenoids and anthraquinones were only detected in methanolic extract. Nonetheless, protein-xanthoprotein and sterols were not present in both extracts. The assorted phytochemicals are common compounds to give pharmacological benefit. However, there were certain compounds present in this herb are likely to be different from the other plants. Therefore, they can be recommended to be used as therapeutic agent to certain illnesses.

Even so, *Zea mays* hair found in *Combatore* has

Table 1. Qualitative analysis of the phytochemicals of Malaysian *Zea mays* hair extracts

Constituent	Aqueous extract	Methanolic extract
Phenols	+	+
Flavonoids (Test I & II)	+	+
Tannins	+	+
Phlobatannins	+	+
Alkaloids (Test I, II & III)	+	+
Terpenoids	-	+
Saponins	+	+
Sterols	-	-
Protein-xanthoprotein	-	-
Cardiac-glycosides	+	+

+ = Present; - = Absent

been reported to contain flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, terpenoids and tannins (Thoudam *et al.*, 2011). Dubiously, saponin was detected in both Malaysian *Zea mays* extracts. While in *Zea mays* husk, only phlobatannins, tannins, polyphenols and steroids were detected (Owoyele *et al.*, 2010). Yet, *Saccharum spontaneum* a different species but from the same family of Gramineae consist of alkaloids, saponins, flavonoids, sugar and tannins (Vhuiyan *et al.*, 2008). Whereas, *Triticum aestivum* which also from the same family containing alkaloids, saponins, carbohydrates, amino acid and protein (Ashok, 2011).

The TPC of aqueous extract was found to be significantly higher ($42.71 \pm 0.87 \mu\text{g/g}$ of TAE) than the methanolic extract ($40.38 \pm 1.10 \mu\text{g/g}$ of TAE). This result showed the aqueous extract gave higher recovery of tannins compared to methanolic extract as claimed by Humadi and Istudor (2009). However, *Zea mays* hair extracts had a conspicuously low TPC value compared to barley, from the same family which was $387.33 \mu\text{g/g}$ of TAE (Oueslati *et al.*, 2009).

The low TPC of the *Zea mays* hair was influenced by a various parameters. Extraction process involved separation of active fractions from plant tissue by using selective solvents and extraction methods (Das *et al.*, 2010). Though the solvent used was different in each method, it had influenced the phenols recovery in the extracts. Since the boiling point of water higher than methanol, the aqueous extract yield was better than methanolic extract (Hodzic *et al.*, 2009). Furthermore, the phenols would dissolve better in the solvents with similar polarity (Green, 2004). The aqueous extract thus contained higher phenols which diffused more in water compared to the methanol solvent.

The limitation of the study was the *Zea mays* hair part used. In this study, inner hair was used despite of the outer hair due to it hygiene and health features. Despite the fact that, the outer layer of *Zea mays* hair most probably contains better yield of anthocyanin based of its fragrant reddish colour. Whereas to the inner hair has yellowish colour, which is known as

flavones and flavanols (Sumati *et al.*, 2006). Above of all, phenols have a high affinity to chelate metals and scavenge the free radical in cells (Michalak, 2006).

Still, there are primary factors influencing the variability of phytochemicals in plants comprising genotype, size and maturity, soil conditions, fertilization, irrigation, pesticide utilization, disease and pests, location and climate, and season (Xin *et al.*, 2006). Thus, these factors can be applied to improve and enhance phytochemical content in plants.

However there are possibilities for the presence of other compounds in *Zea mays* hair. Sugar and carbohydrates may as well presence in *Zea mays* hair due to the sweet odour released during concentrating the filtrate. However a tests need to be carrying out to prove the presence of sugar and carbohydrates as well as steroids, anthraquinones and amino acid.

Conclusions

Zea mays hair extract contains of flavonoids, saponins, tannins, phlobatannins, phenols, alkaloids, and cardiac glycoside. However, only terpenoids were detected in methanolic extract. In addition, the TPC in aqueous extract was higher compared to methanolic extract as the values were $42.71 \pm 0.87 \mu\text{g/g}$ and $40.38 \pm 1.10 \mu\text{g/g}$ of tannic acid equivalent, correspondingly. Therefore the results suggested that bioactive compounds found in *Zea mays* hair, contribute to various pharmaceutical responses as claimed previously. Hence, further study need to be conducted to explore the benefits and the ability of *Zea mays* hair extracts as one of the therapeutic raw material in food, nutraceutical and pharmaceutical industries.

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