Assessment of Malaysian Cornsilk Bioactive Compounds and Its Cytotoxicity test on Brine Shrimp (*Artemia salina*)

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ABSTRACT: Cornsilk contains various beneficial phytochemical compounds. There are claims that cornsilk exhibits various biological activities *in vivo*. The aim of the present study was to determine the cornsilk bioactive compounds and the cytotoxicity effect of cornsilk extract on brine shrimp. Cornsilk was extracted using different solvents with single extraction procedures. All phytochemical compounds samples were identified via GC-MS equipment. The brine shrimp toxicity test was observed to obtain LC_{50} value for both extracts. Eleven compounds were identified in the aqueous extract of cornsilk (AEC) while methanolic extract of cornsilk (MEC) contained 4 compounds. These compounds consist of cyclopentane-ethyl, 2-methylheptane, furfural, furfuryl alcohol, furfuryl-5-methyl, 1-naphtol, lactone-G, cis-2-pentenal, pyranone, hydroxymethyl furfural and palmitic acid. There was only 1 similar compound found in both extracts which was the hydroxymethyl furfural. Cytotoxicity test showed that cornsilk is non toxic. The LC_{50} values found in AEC and MEC were 3151.34 and 1350.65, respectively. Our result found that cornsilk contained various bioactive compounds which positively supported most of the therapeutic activity claimed. In brief, the cytotoxicity test shows that cornsilk is non toxic for human consumption due to its high LC_{50} value.

Keywords: cornsilk, brine shrimp mortality, bioactive compound, cytotoxicity test

Introduction

Zea mays is a species of *Zea* genus and it belongs to tribe Andropogoceae. The subfamily is Panicoideae and family is Poaceae (USDA, 2005). There are 5 species of *zea* genus, which *Z.*

diploperennis, *Z.luxurians*, *Z. mays* L., *Z. nicaraguensis* and *Z. parennis*. However, *Z. mays* subspecies of *mays* is the only cultivated species. Other species and subspecies are wild grasses and referred as teosintes. *Z. mays* is a native plant in North America and is widely grown around the world in medium to high climate countries. The name *Zea* is originated from Greek which means cereal or grain. The ephitet *mays* is believed to be derived from the native Arawak word *maiz* or *mahiz* to describe the plant in America. The word was taken over by the Spanish crew of Columbus's first voyage who first picked up the grain and brought it to Europe (Hyam and Phankhurst, 1995; Desjardin and McCarthy, 2004).

Cornsilk is found inside the husk and hardly revealed until the emergence of yellow pale silk at the end of the husk. This herb has been reported to have properties of antioxidant (Liu *et al*., 2011), antiprostatitis and antispasmodic (Buhner, 2007). In addition, cornsilk is well known in treating infection and cystitis (Steenkamp, 2003), kidney stone and other renal illness (Maksimovic *et al*., 2004). In addition, cornsilk was also reported to contain sitosterol, stigmasterol, fatty and volatile oil, saponin, glucoside substance, vitamin C and K. In a previous study, cornsilk was reported to consist of saponin, terpenoid, glycoside, alkaloid, tannin and phlobatannin, flavonoids and phenol (Solihah and Wan Rosli 2012). In addition, cornsilk was reported to be rich in phenolic compounds, particularly flavonoids (Maksimovic *et al*., 2004).

Cornsilk is an abandoned portion of corn. In Malaysia, cornsilk is completely discarded upon harvesting and is not practiced as traditional remedies. In some parts of other countries namely, North America, Philippine and China, the used of cornsilk is very popular in biological (Kim *et al*., 2005), pharmaceutical, food and beverage (Hasanudin *et al*., 2012), as well as in cosmetics industries (Revlon Con. Products, 2002). There were many claims of therapeutic effects of cornsilk including anti-diabetic (Guo *et al*., 2009), gout, cystitis, chronic nephritis (Maksimovic *et al*., 2004, Tahraoui *et al*., 2007), anti prostatitis and anti-spasmodic activity (Buhner, 2007).

In the last few years, gas chromatography mass spectrometry (GC-MS) has firmly established as a key technological platform for identification and determination of bioactive or phytochemical compounds in both plant and non-plant species. Generally, to extract functional bioactive compounds, various organic solvents with different polarity have been used such as chloroform, chloromethane, hexane, ethyl acetate, ethanol, methanol and water. Extraction and isolation of bioactive compounds from natural products are performed due to the increased demand of using

herbal medicines over the years. Users assume herbal medicines are safe and harmless as they are natural and traditionally used. Thus, it is important to carry out toxicity studies and determine the safety of herbal products (Hasanudin *et al*., 2012). Recently, subchronic study using male and female Wistar rats revealed that cornsilk is non-toxic in nature (Wang *et al.*, 2011). However, the cytotoxicity evaluation of Malaysian cornsilk has not been reported yet.

The biological activities of crude extract of cornsilk are mainly due to the presence of bioactive compounds. Meanwhile, cytotoxicity screening of brine shrimp is a fundamental procedure to assess the plant toxicity. This procedure required less amount of toxin, inexpensive and very simple (Krishnaraju *et al*., 2005). For that reason, the determination of volatile compounds and cytotoxicity effect of crude extracts of Malaysian cornsilk on brine shrimp were evaluated.

Materials and methods

Plant material

Corn fruit was bought from local wet market at Pasar Siti Khatijah located in Kota Bharu town (Kelantan, Malaysia). Cornsilk was collected and dried in an oven over night at 55^{o}C until golden yellowish color was obtained. Dried cornsilk was then ground into powder using a domestic grinder (National; MX-895).

Preparation of extracts

Dried cornsilk powder was boiled for 30 min with distilled water (1:15, w/v) to get the aqueous extract of cornsilk (AEC). Meanwhile, the methanolic extract of cornsilk (MEC) was prepared using a Soxhlet apparatus (1:4, w/v). Both AEC and MEC were prepared according to method from Solihah and Wan Rosli (2012). In order to identify the presence of bioactive compounds, the AEC were subsequently extracted using different solvents as described below.

Chloroform and dichloromethane

Ten (10) ml of AEC was extracted with 10 ml of chloroform in 50 ml separation funnel. The funnel was shaken in the fume hood for 20 times and the air was released. This procedure was repeated

again. Later the extract was left at room temperature to form immiscible layers. The glass rod was used to aid the formation of immiscible layers by stirring between the aqueous and organic layers. The bottom layer was then collected. For the other fraction, the upper layer was re-extracted with fresh solvent following the same step as described earlier. Later, the bottom layer was combined and concentrated to 1 ml using a vacuum evaporator (Heidolph, Laborota 4000). The sample was kept at -20^{o}C prior to injection into GC-MS. Another batch of AEC (10 ml) was extracted with DCM and followed the similar procedures as described above.

Hexane and ethyl acetate

Ten (10) ml of AEC was extracted with 10 ml of hexane in 50 ml separation funnel. The funnel was gently shaken in the fume hood for 20 times and the pressure was released. The step was repeated again. Later the extract was left to form immiscible layers. The glass rod was used to aid the forming layers by stirring between the aqueous and organic layers. The top layer was collected while the bottom layer was re-extracted with fresh solvent following the same step as described before. Later, the top layer was combined and concentrated to 1 ml using a vacuum evaporator (Heidolph; Laborota 4000). The sample was kept at -20^{o}C prior to injection into GC-MS. Another batch of AEC (10 ml) was extracted using ethyl acetate and followed the similar procedures as described earlier.

Dissolving AEC and MEC with Methanol

About 500 mg crude AEC was dissolved in 5 ml of methanol. The extract was filtered using membrane filter (Sartorius, 0.45 µm). The filtrate was concentrated to 1.0 ml using a vacuum evaporator (Heidolph, Laborota 4000) and kept at -20^{o}C prior to injection into GC-MS. This procedure was repeated using crude MEC as described earlier.

Determination of Bioactive Compound of Cornsilk via GC-MS

All samples were analyzed by using GC-MS (Perkin Elmer, Clarus 600T-MS) which was equipped with a mass spectral detector. All extracts were run using the ELITE-5-MS, methylpolysiloxane (5% Phenyl) column (30m x 0.25mm id, film thickness 0.25mm, Perkin Elmer, USA). A split flow of 20:1 was used for these samples. One µl of the sample was injected into the GC injection port.

The oven temperature was increased from 60 to 230^{o}C at a rate of 3^{o}C /min and held for 12.33 min. The injector and detector temperatures were set at 250^{o}C. The helium carrier gas flow rate was 1.5 ml/min. The scanning mass range *(m/z)* was from 28 to 500 Da and the ionization energy was 70 eV.

Brine shrimp assay

The egg of brine shrimp was hatched for 48h in a beaker containing 200 ml sea water. This beaker was placed under bright light during the hatching process. Brine shrimp larvae were separated from the egg and placed into another container containing artificial sea salt water. The toxicity test was performed according to Wanyoike et al. (2004). Ten brine shrimps were placed in a vial which contained 5 ml of sea salt. The concentration of sea salt was reduced in line with the increased of AEC and MEC concentration at 10, 100 and 1000 µg/ml, respectively to total up to 5 ml. Each concentration was performed in 5 replicates and the survived brine shrimps were identified using magnifier glass.

The result of mortality (%) was calculated from the mean survival brine shrimp. Median lethality concentration (LC_{50}) was obtained from the best-fit line plotted from the log concentration versus percentage of mortality (Eq. 1).

$$\text{Mortality (\%)} = (\text{Death larvae} / \text{Total larvae}) \times 100 \qquad \text{(Eq. 1)}$$

Statistical Analysis

The results were analyzed using IBM SPSS Statistics Version 19. Data were presented as mean values of three replicates ± standard deviation (SD) which were subjected to one-way ANOVA. For comparison of means, Tukey HSD was used and significant difference was determined at $p<0.05$.

Results and Discussion

Bioactive compounds of cornsilk

Ten compounds were identified in AEC. Meanwhile only 4 compounds were identified in the MEC. All the compounds were illustrated in **Table 1**. The only similar compound identified in both extracts was 5-hydroxymethyl, furfural.

Acute mortality of crude extracts

The lethality rate of AEC, MEC and distilled water were shown in Figure 1. Meanwhile Table 2 illustrated the LC_{50} of AEC and MEC, respectively. Figure 1 shows the mortality rate of brine shrimp larvae when they were exposed to test solution over 24h. All brine shrimp larvae exposed to distilled water (negative control) over 24h survived. While for exposing the brine shrimp larvae to 5000 ppm of AEC, a low mortality was observed. However, the mortality rate increased to 80 % when the brine shrimp larvae were exposed to 7000 ppm of AEC and then total mortality occurred when the concentration of AEC increased over 9000 ppm. On the other observation on MEC exposure to brine shrimp larvae, the mortality was observed at a concentration as early as 10 ppm, though it was low (4%). Furthermore, the total mortality of brine shrimp larvae exposed to MEC (7000 ppm) occurred earlier at a lower concentration than AEC (9000 ppm). The LC_{50} of AEC was 2 times higher than the value of MEC.

Table 1: Bioactive Compound Detected in Cornsilk Extracts using Different Solvents

Extract	Extraction solvent	RT*	Compound name	Area (%)
AEC	Hexane	1.79	Cyclopentane, ethyl	5.62
		1.96	2-methylheptane	1.67
	Methanol	2.71	Furfural	1.63
		3.00	Furfuryl alcohol	1.17
		5.03	Furfuryl, 5-methyl	0.98
		18.23	1-naphtol	1.67
		24.92	Lactone-G	2.31

	Dichloromethane	4.47	Cis-2-pentenal	0.17
	Ethyl acetate	10.98	Pyranone	0.34
		14.59	5-hydroxymethyl, furfural	0.90
	Chloroform	41.87	Palmitic acid	0.17
MEC	Methanol	1.78	Acetic acid	4.27
		13.42	4-methyl itaconate	1.53
		14.59	5-hydroxylmethyl, furfural	0.90
		31.86	3-deoxy-d-mannoic lactone	2.27

*Retention time

Acute mortality of crude extracts

The lethality rates of AEC, MEC and distilled water were shown in **Figure 1**. Meanwhile **Table 2** illustrated the LC_{50} of AEC and MEC, respectively.

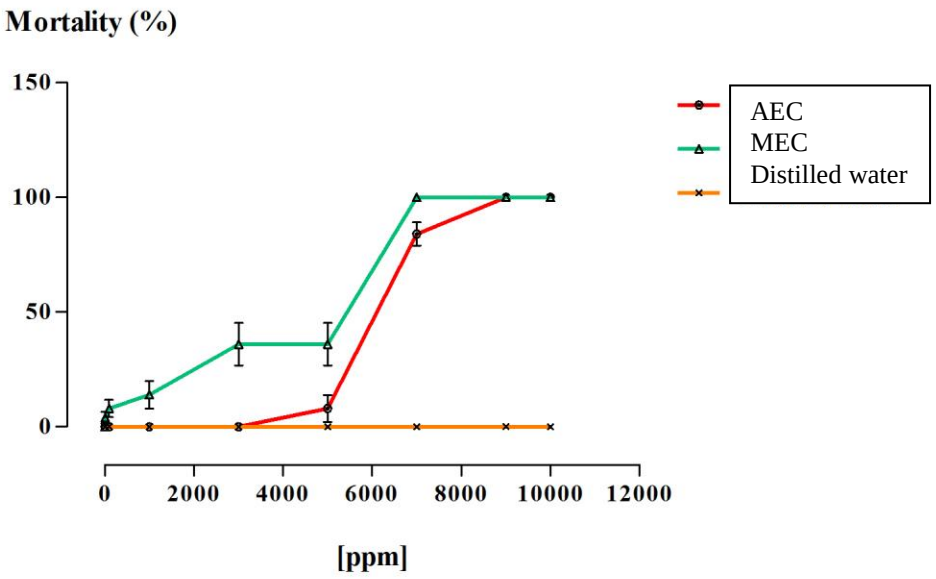

Figure 1: Mortality rate of cornsilk extracts

Table 2: The LC_{50} of brine shrimp in cornsilk extracts

	Survival of brine shrimp (%)									**LC_{50}**
[µg/ml]	**0**	**10**	**100**	**1000**	**3000**	**5000**	**7000**	**9000**	**10000**	
AEC	100	100	100	100	100	70	20	0	0	3151.34
MEC	100	96	92	86	64	64	0	0	0	1350.65

Discussion

Hexane was used to extract the lipid compositions from AEC. The component of lipid identified by GC-MS was cyclopentane, ethyl and 2-methylheptane. Information on the biological activity of cyclopentane, ethyl itself was rather scanty. However, the cyclopentane ring could demonstrate various biological activities due to their ability to synthesize prostaglandin especially in pharmacological and medicinal characteristics. Biaggio *et al.* (2005) found that prostaglandin analogs contain heteroatoms in the cyclopentane ring. It is recognized in regulating the contraction or relaxation of smooth muscle on the vascular and non vascular system (Nakano, 1972). In addition, Chand *et al.* (2001) has reported that related activity of cyclopentane derivative was found to exhibit anti influenza activity. Another compound identified was an alkane group, namely 2-methylheptane. Its specific contribution to biological activity was unknown, however this unbranched alkane most possibly originated from lipid oxidation (Raitio *et al.*, 2011).

Semi polar compound was extracted using methanol. There were 5 compounds identified using methanol that consist of 3 furan derivatives, 1-naphtol and lactone-G. Furan and its derivatives were products of thermal degradation including from the Maillard reaction and caramelization (Ait Ameur *et al.*, 2008). Furfural and furfuryl alcohol exist naturally in bread, coffee, baked products and essential oils. El-Moughy *et al.* (2008) reported that furfural was helpful in reducing fungicidal and nematicidal activities. Adams *et al.* (1997) clarified that furfural was used as a flavour ingredient. On the other hand, the information of 1-naphtol activity was insufficient. Though, Fujiwara *et al.* (2008) reported that 1-naphtol acts as an inhibitor substance in glucuronidation in human liver microsomes.

Lactone G was a sesquiterpene. It structure has three isoprene units with a lactone ring. There was scarce information related to lactone G. However, lactone in general, was reported to form from a

biosynthesis pathway which involved hydroxyacid cleavage of lipid (Heath and Reineccius, 1986). Lactone can be found in matured apricot and stone fruit (Aubert *et al.*, 2010; Greger and Schieberle, 2007). Besides that, dichloromethane and chloroform are well known for their capability in extracting lipid profiles. Cis 2-pentenal and hexadecanoic acid were identified in the dichloromethane and chloroform extracts, respectively. Cis 2-pentenal can be found in virgin olive oil (Angerosa *et al.*, 2004) and strawberries (Hui, 2010). Cis 2-pentenal was very rare compared to trans 2-pentenal. Besides that, their biological activity detail was also unclear. However, Canonero *et al.* (1990) reported that cis 2-pentenal was formed from lipid peroxidation. Meanwhile, palmitic acid is commonly found in fruits and nuts. These compounds are commercially used as flavoring agent, detergent and cosmetic formulas. Harada *et al.* (2002) reported that hexadecanoic acid found in marine red alga exhibited antitumor activity in mice. Whereas Yff *et al.* (2002) found hexadecanoic acid to exhibit antibacterial activity. Lately, Aparna *et al.* (2012) reported that hexadecanoic acid also showed anti-inflammatory activity. It was meticulously used as medicated oil to treat rheumatic symptoms. On the contrary, according to the World Human Organization (2003), excessive consumption of hexadecanoic acid may cause the development of cardiovascular disease.

Ethyl acetate, a polar solvent was used to extract the compounds in AEC. Two compounds identified as pyranone and 5-hydroxymethyl, furfural were actually the flavonoid and furan derivatives, correspondingly. Generally, pyranone can be found mostly in fungi, wine and grape (Cutzach *et al.*, 1997). It has antifungal activity on pathogenic filamentous fungi or pathogenic yeast (Komai *et al.*, 2003). Other than that, this compound was also claimed to show antimicrobial and anti-inflammatory activities (Gopalakrishnan and Vadivel, 2011). Whereas 5-hydroxymethyl, furfural often associates with better quality honey and also can be found in infant formulas (Ferrer *et al.*, 2002) and bakery products. de Souza *et al.* (2012) reported that this compound was formed from carbohydrate namely glucose and fructose, while Kenney and Bassette (1959) reported that it was derived from hexose degradation.

MEC itself contained acetic acid, 4-methyl itaconate, 5-hydroxymethyl, furfural and 3-deoxy-d-mannoic lactone. Schwan and Wheals (2004) reported that acetic acid was a product from the oxidation of ethanol during fermentation. On the other occasion, Rivard and Grohmann (1991) reported that acetic acid was formed from the furfural breakdown. Kalua *et al.* (2007) reported that

carboxylic acid often associated with microbial fermentation and other fruit handling defects. There was specific bioactivity reported on acetic acid.

There is scanty information about 4-methyl itaconate compound. However, a study performed by Santen Pharmaceutical Co., Ltd (2000) found that itaconic acid derivatives were used as a substitute component of polymer. Most of the natural monomers have been used in industrial application of chemical engineering, pharmaceuticals, food and agriculture due to the lower cost, biodegradable characteristics, free availability and non toxicity (Shastri, 2003). The other compound, 3-deoxy-d-mannoic lactone was a cyclic ester with unknown potential bioactivity. However, this compound has been identified in garlic (Shobana *et al.*, 2009) and polypore mushroom (Teoh and Mashitah, 2012).

The brine shrimp toxicity test is considered a rapid screening to examine the toxicity of a crude extract. This screening technique justifies the observed mortality rate of the extract. A lower LC_{50} value shows the higher toxicity of an extract. The LD_{50} values of AEC and MEC were at 5545.45 and 4304.09 µg/ml, respectively. Both extracts showed higher LD_{50} values. According to Musila et al. (2013), natural plant that possesses $LC_{50} \leq 500$ µg/ml is considered toxic to the brine shrimp larvae. Meanwhile, Meyer et al. (1982) reported that $LC_{50} \leq 1.0$ mg/ml of extract was toxic. However in this study, classification of toxicity was determined according to Deciga-Campos et al. (2007). There are 3 levels of toxicity consist of toxic ($LC_{50} < 500$ µg/ml), less toxic ($500 \leq LC_{50} \geq 1000$ µg/ml) and non toxic ($LC_{50} > 1000$ µg/ml). Thus the present study revealed that Malaysian cornsilk extract was nontoxic to the brine shrimp larvae.

The differences between LC_{50} of both extracts were notable. The MEC showed the higher lethality concentration possibly due to the solvent itself. Musila et al. (2013) have reported before regarding the extremely high differences of lethality of organic extract compared to aqueous. This incident might be due to the presence of methanol traces in the MEC, although it was expected to be completely removed using vacuum evaporator.

Conclusion

This present result supports the used of cornsilk as traditional therapeutic herbal item for health and medicinal purposes. Cornsilk contains various bioactive compounds which positively support most

of the therapeutic activity claimed. In brief, the cytotoxicity test shows that Malaysian cornsilk is non toxic for human consumption due to its high LC_{50} value. Thus, it has the potential to be used as functional pharma-nutritional ingredient in food and pharmaceutical products.

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