UNIVERSITI SAINS MALAYSIA
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LAPORAN AKHIR

DEVELOPMENT OF DIABETIC-FRIENDLY JUICES DEVELOPED FROM CORNSLIK EXTRACT AND STABILITY OF ITS ANTIOXIDATIVE POLYPHENOLIC COMPOUNDS

PENYELIDIK

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PENYELIDIK BERSAMA

DR. WAN AMIR NIZAM WAN AHMAD

2015

UNIVERSITI SAINS MALAYSIA — USM — APEX	RU GRANT FINAL REPORT FORM

Please email a softcopy of this report to rcmo@usm.my

A	PROJECT DETAILS
i	**Title of Research:** DEVELOPMENT OF DIABETIC-FRIENDLY JUICES DEVELOPED FROM CORNSILK EXTRACT AND STABILITY OF IT ANTIOXIDATIVE POLYPHENOLIC COMPOUNDS
ii	
iii	**Name of Research Leader: Wan Rosli Wan Ishak @ Wan Ahmad (Assoc Prof)**
iv	**Name of Co-Researcher:** 1. Dr Wan Amir Nizam Wan Ahmad
v	**Duration of this research:** **Start Date** : Oct 1, 2012 a) **Completion Date** : 30 Sept 2015 b) **Duration** : 3 years c) **Revised Date** (*if any*) : ....

B	ABSTRACT OF RESEARCH
	(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large) This study aims to develop diabetic-friendly juices developed from cornsilk extract and to investigate stability of it antioxidative polyphenolic compounds. The rats with different dose treatments of cornsilk extract ranging from 400 to 800 mg/kg. Cumulative urine of aqueous extract of cornsilk (AEC) at 400 mg/kg (14.06 ml) and 500 mg/kg (15.21 ml) treatments found to be significantly lower than positive control (21.25 ml). The ED_{50} of AEC was observed at 454.10 mg/kg. Malaysian AEC had shown a mild diuretic activity in elevating urine and Na^+ content at dosages from 500 to 800 mg/kg. Whilst, AEC also showed an effect of potassium sparing diuretics. The highest content of total polyphenol of immature silks was exhibited by ethanol extract (92.21 mg GAE/g) while water extract (64.22 mg GAE/g) had the highest polyphenol content among mature silk extracts. In ABTS assay, all immature silk extracts had higher percentage of inhibition compared to the mature silks. The ethanol extract of immature (EC50 = 0.478 mg/ml) and mature silk (EC50= 0.799 mg/ml) exhibited the strongest antioxidant capacity followed by the water and ethyl acetate extract. Cornsilk extracts are potential ingredient to be applied in food industries and at the same time reducing agriculture wastage.

Abstrak

Kajian telah dilakukan untuk menghasilkan jus yang diformulasikan daripada ekstrak sutera jagung muda dan menentukan kestabilan sebatian antioksidatif polifenol. Tikus kajian telah diberi rawatan ekstrak sutera jagung pada dos yang berbeza dalam julat antara 400 hingga 800 mg/kg. Urin kumulatif ekstrak akues sutera jagung (AEC) pada dos 400 (14.06 ml) dan 500 mg/kg (15.21 ml) didapati lebih rendah daripada kawalan positif (21.25 ml). Nilai ED_{50} AEC didapati sebanyak 454.10 mg/kg. AEC Malaysia telah menunjukkan aktiviti diuretik sederhana dalam meningkatkan kandungan urin dan Na^+ pada dos 500 hingga 800 mg/kg. Sementara itu, AEC juga menunjukkan kesan penahanan diuretik. Kandungan polifenol paling tinggi dikesan dalam sutera jagung muda dikesan dalam ekstrak etanol (92.21 mg GAE/g) manakala ekstrak berair (64.22 mg GAE/g) menunjukkan kandungan polifenol paling tinggi antara ekstrak sutera jagung matang. Dalam esei ABTS, semua ekstrak sutera jagung muda menunjukkan peratusan perencatan radikal bebas yang tinggi berbanding sutera jagung matang. Ekstrak etanol sutera jagung muda (EC50 = 0.478 mg/ml) dan sutera jagung matang (EC50= 0.799 mg/ml) menunjukkan kaupayaan kapisiti pengoksidaan yang paling tinggi diikuti oleh ekstrak berair dan etil asetat. Ringkasnya,ekstrak sutera jagung berpotensi sebagai ramuan makanan untuk diaplikasikan dalam industri makanan dan dalam masa yang sama mengurangkan pembaziran pertanian.

C BUDGET & EXPENDITURE

i

Total Approved Budget : RM 207 450

<u>**Yearly Budget Distributed**</u>

Year 1 : RM

Year 2 : RM

Year 3 : RM

Total Expenditure : RM 192 642.39

Balance : RM 14 807.61

Percentage of Amount Spent (%) : 92.86

 # Please attach final account statement (eStatement) to indicate the project expenditure

ii Equipment Purchased Under Vot 35000

No.	Name of Equipment	Amount (RM)	Location	Status
	na			

 # Please attach the Asset/Inventory Return Form (Borang Penyerahan Aset/Inventori) – Appendix 1

D RESEARCH ACHIEVEMENTS

i	**Project Objectives** *(as stated/approved in the project proposal)*

No.	Project Objective	Achievement
1	To determine nutritional composition of both cornsilk from immature and mature corn	yes
2	To determine the concentration of polyohenolic compounds before processing and upon processing	Yes
3	To elucidate the antioxidative activities and scavenging capacities of cornsilk extract	Yes
4	To investigate the diuretic activities of cornsilk extract in normal rats	Yes
5	To develop cornsilk juice and investigate the changes in physico-chemical properties of cornsilk juice during and after processing	Yes

ii	**Research Output**

a) Publications in ISI Web of Science/Scopus

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1	Nutritional Compositions and Antioxidant Capacity of the silk Obtained from Immature and Mature Corn. Nurhanan, A. R. Wan Amir Nizam WA and Wan Rosli WI*. Journal of King Saud University (Science), 2014, 26: 119-127. SCOPUS.	PUBLISHED
2	Aqueous Extract of Cornsilk Confers Mild Diuretic Activity in Normal Rats. Solihah, M. A., Nurhanan, A. R. and Wan Rosli, W. I* and Wan Amir Nizam WA. SAINS MALAYSIANA 44(8)(2015): 1167-1174. ISI	PUBLISHED
3	Assessment of Bioactive Compounds and Cytotoxicity test of Malaysian Cornsilk and on Brine Shrimp (*Artemia salina*). Wan Rosli WI* and Solihah MA. Health and the Environment Journal. Accepted for publication on Sept 30, 2014.	In Press
4	Antioxidative Activities and Polyphenolic Content of Different Varieties of Malaysian Young Corn Ear and Cornsilk.. Ho YM, Wan Amir Nizam WA and Wan Rosli WI*. SAINS MALAYSIANA. ISI	In Editing by SAINS MALAYSIANA

b) Publications in Other Journals

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)

c) Other Publications
(book,chapters in book,monograph,magazine,etc.)

d) Conference Proceeding

No.	Conference (conference name,date,place)	Title of Abstract/Article	Level (International/National)
1	4th Malaysian Symposium on Biomedical Sciences, 11-12 May 2013. Venue: School of Medical Sciences USM.	Effect of aqueous extract of cornsilk on glycemic index in STZ-induced diabetic *Sprague-Dawley* rats	National
2	3rd International Wellness, Health Lifestyle and Nutrition, 12-14 Dec 2012. Venue: School of Health Sciences USM.	Determination of ascorbic acid in Malaysian cornsilk using HPLC	International
3	Poster presentation at 29th Annual Scientific Conference 2014 organized by Nutrition Society of Malaysia. Reneaissance Hotel, Kuala Lumpur. June 2014.	Antioxidant capacity, total polyphenol and total flavanoid content in young corn ear and cornsilk of different varieties	National

 # Please attach a full copy of the publication/proceeding listed above

iii Other Research Ouput/Impact From This Project
(patent, products, awards, copyright, external grant, networking, etc.)

1. **Award:**
   Third Prize Winner in Poster Presentation in Pharmacology/Toxicology). Siti Fatimah AR, Wan Amir Nizam WA and Wan Rosli WI. Effect of aqueous extract of cornsilk on glycemic index in STZ-induced diabetic Sprague-Dawley rats. 4th Malaysian Symposium on Biomedical Sciences, 11-12 May 2013. Venue: School of Medical Sciences USM.

2. **Trade Mark**
   Registration of Trademark TM (CORNLET) in Class 30 (2014010231 TM) on July 8, 2015.

E HUMAN CAPITAL DEVELOPMENT

a) Graduated Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD	/		1. Nurhanan Abdul Rahman (2015)
MSc	/		1. Solihah Mat Ali (2014)
Undergraduate	/ /		1. Siti Fatimah Ab Rahim (2014) 2. Ho Yoke Mei (2014)

b) On-going Human Capital

Student	Nationality (No.)		Name
	National	International	
MSc			
Undergraduate	/		Development of Beverages from Cornsilk Extract and Its Effect of Sensory and Mineral Composition

c) Others Human Capital

Student	Nationality (No.)		Name
	National	International	
Post Doctoral Fellow			1. 2.
Research Officer			1. 2.
Research Assistant			1. 2.
Others (....................)			1. 2.

F COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

- Introduction
- Objectives
- Methods
- Results
- Discussion
- Conclusion and Suggestion
- Acknowledgements
- References

G PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY

(Please provide issues arising from the project and how they were resolved)

na

H RECOMMENDATION

(Please provide recommendations that can be used to improve the delivery of information, grant management, guidelines and policy, etc.)

na

I	COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S RESEARCH COMMITTEE
	....

Purchase Requisition ▸ Purchase Order ▸ Suppliers ▸ Maintenance ▸ Financials ▸ Coda Info ▸ Reports ▸ Admin ▸

UserCode: MROHAYU / USMKCKLIVE / PPSK **Program Code: Votebook9100** **Current Program : Votebook (Header)**

Current Date : 24/11/2015 9:52:00 AM **Version: 15.120, Last Updated at 10/12/2014** **DB: 13.00, 9/18/2010 VB: 13.01, 3/14/2011** **Switch Language : English / Malay**

Wildcard : eg. Like 100%, Like 10%1, Like %1

Element 1: 1001 Element 2: % Element 4: PPSK

Element 5: 813057 Year: 2015

Detail	Excel	Budget Rule	Budget Control	Account Description	Budget Account Code	Roll over	Budget	Cash Received	Advanced	Commit	Actual	Available	Percentage
Detail	Excel	46	T	Projek Kumpulan Wang Uni Penyelidikan	1001.111.0.PPSK.813057	22,909.89	0.00	0.00	0.00	0.00	0.00	22,909.89	0.00%
Detail	Excel	46	T	Projek Kumpulan Wang Uni Penyelidikan	1001.114.0.PPSK.813057	3,600.00	1,800.00	0.00	0.00	0.00	0.00	5,400.00	300.00%
Detail	Excel	46	T	Projek Kumpulan Wang Uni Penyelidikan	1001.115.0.PPSK.813057	-500.00	0.00	0.00	0.00	0.00	0.00	-500.00	0.00%
		46	T	SubTotal		26,009.89	1,800.00	0.00	0.00	0.00	0.00	27,809.89	1,544.99%
Detail	Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.221.0.PPSK.813057	-10,848.38	5,000.00	0.00	0.00	0.00	6,172.49	-12,020.87	-240.42%
Detail	Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.223.0.PPSK.813057	300.00	150.00	0.00	0.00	0.00	0.00	450.00	300.00%
Detail	Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.225.0.PPSK.813057	-257.47	0.00	0.00	0.00	0.00	185.10	-442.57	0.00%
Detail	Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.227.0.PPSK.813057	12,790.40	800.00	0.00	0.00	1,066.70	718.11	11,805.59	1,475.70%
Detail	Excel	47	T	Projek Kumpulan Wang Uni Penyelidikan	1001.229.0.PPSK.813057	-21,152.97	5,000.00	0.00	0.00	500.00	5,055.46	-21,708.43	-434.17%
		47	T	SubTotal		-19,168.42	10,950.00	0.00	0.00	1,566.70	12,131.16	-21,916.28	-200.15%
Detail	Excel	48	T	Projek Kumpulan Wang Uni Penyelidikan	1001.335.0.PPSK.813057	9,000.00	0.00	0.00	0.00	0.00	0.00	9,000.00	0.00%
		48	T	SubTotal		9,000.00	0.00	0.00	0.00	0.00	0.00	9,000.00	0.00%
Detail	Excel	50	T	Projek Kumpulan Wang Uni Penyelidikan	1001.552.0.PPSK.813057	-86.00	0.00	0.00	0.00	0.00	0.00	-86.00	0.00%
		50	T	SubTotal		-86.00	0.00	0.00	0.00	0.00	0.00	-86.00	0.00%
		9999		GrandTotal		15,755.47	12,750.00	0.00	0.00	1,566.70	12,131.16	14,807.61	116.14%

Journal of King Saud University – Science (2014) 26, 119–127

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ORIGINAL ARTICLE

Nutritional compositions and antioxidative capacity of the silk obtained from immature and mature corn

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Received 24 February 2013; accepted 8 November 2013
Available online 21 November 2013

KEYWORDS
Nutritional compositions;
Mineral;
Antioxidant capacity;
Corn silk;
Immature

Abstract The silks of immature and mature corn were evaluated for their variations in nutritional compositions, mineral content and antioxidant capacity. Both immature and mature silks were good source of nutritional compositions. Immature silks contained significantly higher moisture (89.31%) (fresh basis), lipid (1.27%) and protein (12.96%) content than the mature silk. Mature silks contained higher composition of ash (5.51%), carbohydrate (29.74%) and total dietary fiber (51.25 g/100 g), than the immature silk, but the difference was not significant. In mineral determination, immature silk was rich source of Ca (1087.08 μg/g), Mg (1219.17 μg/g), Cu (5.60 μg/g) and Zn (46.37 μg/g) than the mature silks. In contrast, other minerals such as K (35671.67 μg/g), Na (266.67 μg/g), Fe (4.50 μg/g) and Mn (35.57 μg/g) were found higher in the mature silk. The silks were extracted with ethyl acetate, ethanol and water using the Soxhlet extraction method to determine the polyphenol and ABTS radical scavenging capacity. From this study, the highest content of total polyphenol of immature silks was exhibited by ethanol extract (92.21 mg GAE/g) while water extract (64.22 mg GAE/g) had the highest polyphenol content among mature silk extracts. Total flavonoid content of both immature and mature silks was higher in the water extract at 8.40 mg CAE/g and 2.31 mg CAE/g, respectively. In the ABTS free radical assay method, all immature silk extracts had higher percentage of inhibition compared to the mature silks. Among all three crude extracts, the ethanol extract of immature (EC_{50} = 0.478 mg/ml) and mature silk (EC_{50} = 0.799 mg/ml) exhibited the strongest antioxidant capacity followed by the water and ethyl acetate extract.

1. Introduction

Corn is one of the most widely grown cereal crops in the world and has become the third most important cereal crops other than wheat and rice (Ramessar et al., 2008). Production of corn was reported to increase from 713 thousand metric tons in 2006/2007 to nearly 820 thousand metric tons in 2010/2011 (USDA-FAS, 2008). The high production corresponds

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to increased consumption of corn crop when the world consumption of corn was reported to increase rapidly in 2007/2008 and in the following years (USDA-FAS, 2008).

Corn and its other plant parts are used in various food, agricultural and health applications (Naqvi et al., 2011; Voca et al., 2009). Corn cereal is being a significant staple food for some countries. It contains nutritious components essential for health. Its pericarp contains high crude fiber (about 87%) which constituted with 67% hemicellulose, 23% cellulose and 0.1% of lignin. Its endosperm is rich in starch (87.6%) and protein (8%). The germ however is characterized by high composition of crude fat (33%), protein (18.4%) and minerals (Lunven, 1992). The immature corn (baby corn) contains high protein (25.58%) and dietary fiber (30.4%) content and lower content of crude lipid (3.67%) and 3.74% of ash and appreciable total sugar content (10.07 g/100 g) (Rosli and Anis, 2012). On the other nutrient, immature corn contained 5.43 mg/100 g of ascorbic acid amino acid (0.05 g/g methionine, 2.85 g/g isoleucine and 0.6756 g/g leucine). In addition, some minerals such as Ca, Mg and P were presented at 95, 345 and 898.62 mg/100 g, respectively (Hooda and Kawatra, 2013).

Corn silks are a bundle of silky, long and yellowish strands which could be seen on top of both baby corn and corn fruit. The immature part of a corn plant called baby corn is an unfertilized female flower which is usually harvested after 60 days of planting. Conversely, a corn fruit is developed from a fertilized female flower that is later grown into a corn fruit after 4 months of planting. Corns have been used extensively as foods, feeds and are processed to produce oil, starch and ethanol (Ramessar et al., 2008).

Meanwhile, corn silk is predominantly discarded together with other parts of the plant due to lack of effective utilization. The silks function as a stigma of a female flower and as the fruit develops, the silk elongates beyond the cob covering the edible plant part. Traditionally, the hairs are used as a diuretic agent to ease the passing of stones or gravel in kidneys and urinary bladder (Maksimovic and Kovacevic, 2003). In China, corn silk is well known as an important traditional Chinese medicine in treating several illnesses related to kidney (Zhao et al., 2012). Besides, it also was used to treat edema, cystitis, gout, treat rheumatism and rheumatoid, arthritic and as an antimicrobial agent as well (Maksimovic and Kovacevic, 2003; Velazquez et al., 2005; Fatima et al., 2012). Scientifically, corn silk has been reported to exhibit positive effect on glycemic metabolism by increasing the insulin level whereby the increasing of insulin level and recovery of β-cells were known to be the mechanism involved in the glycemic metabolism (Guo et al., 2009). In other study corn silk polysaccharides were found to exhibit an anti-diabetic effect on streptozotocin (STZ)-induced diabetic rats. The daily treatment of 100–500 mg/kg body weight of the polysaccharide had significantly decreased the blood glucose level and serum lipid. Evidently, the total cholesterol level of the polysaccharide treated group of streptozotocin-induced diabetic rats was found significantly lower compared to the normal and control group (Zhao et al., 2012).

It is known that an antioxidant can inhibit or delay oxidation process although at lower concentration than the biomolecule it is protecting (Halliwell, 1995; Gutteridge and Halliwell, 2010). Utilization of synthetic antioxidants as food preservatives has raised consumers' consciousness toward health due to some adverse effects. For instance, an excessive use of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) is harmful and accompanied with side effects thus restricted their uses (Ningappa et al., 2008). Natural antioxidants thus are being studied extensively with regards to confer many benefits to health rather than the harmful effects. Other than health implications, natural antioxidants have advantages due to their functionality such as solubility (Moure et al., 2001). The uses of natural antioxidant are varied. Tocopherols have strong capacity to inhibit lipid oxidation and are used in foods while gallic acid derived from *Peltiphyllum peltatum* has shown nephroprotective effect (Nabavi et al., 2013).

Plants are rich sources of polyphenols and can act as antioxidant. In addition, the harmful effects of free radicals which are known to be associated with several diseases such as cardiovascular disease, diabetes mellitus, cancer, arthrosclerosis, neurodegenerative disease and ageing can be prevented with sufficient intake of polyphenols in the diet (Sugamura and Keaney, 2011; Valko et al., 2007; Williams and Spencer, 2012). These secondary plant metabolites are not only widely distributed in fruits (Zaouay et al., 2012), vegetables (Wootton-Beard and Ryan, 2011), cereals (Qingming et al., 2010), leaves (Costa et al., 2009) and roots (Fu et al., 2012) but the underutilized plant sources like seeds (Tomaino et al., 2013), peels (Wang et al., 2012; Madhava Naidu et al., 2011) and bran (Zhou and Yu, 2004) have been reported to exhibit strong antioxidative effects.

However, the information regarding the nutritional composition such as proximate and mineral composition and antioxidant capacity of the silks from immature and mature corn are still lacking. Therefore, the objectives of this study were to determine moisture, protein, lipid, and total dietary fiber of silks obtained from these two types of silks and to determine the antioxidant capacity of organic and aqueous extracts of these silks as comparison with Trolox standard.

2. Materials and methods

2.1. Sample

Baby corn and sweet corn were purchased from the local wet market in Kota Bharu district, Kelantan state of Peninsular Malaysia. The husks were eliminated and silks were detached from its fruit. Fresh corn silks were chopped and dried in an oven (Memmert, Germany) at 55 °C for 2 days. Dried corn silks were kept in a sealed plastic bag at 4 °C. Prior to analysis, the corn silk was ground into powder form (Waring Commercial, USA).

2.2. Reagents and standards

Folin–Ciocalteu reagent, gallic acid, (+)-catechin, and butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich (GmbH, Sternheim, Germany). Kalium-hexacyanoferrat ($K_3Fe(CN)_6$, 2,2′-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid ($ABTS^+$), Trolox, sulfuric acid, iron (III) chloride anhydrous ($FeCl_3$), nitric acid (65%) and other chemicals were purchased from Merck (Darmstadt, Germany).

Determination of nutritional composition

ritional composition (moisture, protein, lipid and ash) was rmined according to (AOAC, 1996). Moisture content of h and oven dried silks was determined using an oven-dry nique and expressed as fresh and dry basis, respectively. tein content was determined by using the Kjeldahl method rhardt, Germany) and nitrogen content of samples was tiplied by a factor of 6.25. Lipid content was determined g a Soxhlet apparatus with petroleum ether as the extract- solvent. Total dietary fiber composition of silk was deter- ed by an enzymatic–gravimetric method, using sequential ymatic digestions by thermostable α-amylase, protease amyloglucosidase (Sigma–Aldrich (GmbH, Sternheim, many)).

Mineral composition

ieral content (Ca, Mg, K, Na, Cu, Fe, Mn and Zn) of corn was determined using an atomic absorption spectropho- eter (AAS) (Perkin Elmer Analyst, USA). Samples (0.5 g) e ashed and digested in 2 ml concentrated nitric acid. acid-digests were filtered through ashless filter paper hatman 42) and raised to 10 ml with deionized water.

Extraction

it material (20 g) was extracted with three separate solvents yl acetate, ethanol and water) by the using Soxhlet extrac- i method. Crude extracts obtained were vacuumed evapo- d (Heidolph, Germany) to dryness at 50 °C and kept in a wed cap bottle at −18 °C. The yield of the extract obtained reported as percentage and was calculated as follows:

$$\text{ld}(\%) = \frac{\text{Wa} - \text{Wb (g)}}{\text{Dry plant used (g)}} \times 100 \qquad (1)$$

re Wa = weight of bottle + crude extract (g), = weight of bottle (g).

Determination of total polyphenol content

total phenolic content of extracts was determined using Folin–Ciocalteau method (Kaur et al., 2008). One milliliter rude extract (1 mg/ml) was added with 1 ml of Folin–Cio- eau reagent (1:1) and 1.5 ml of sodium bicarbonate % w/v). The mixture was raised to 10 ml with distilled er. After 2 h of reaction at room ambient the absorbance recorded at 765 nm and used to calculate the phenolic con- by comparing with gallic acid standard. The total phenolic tent was then expressed as mg gallic acid equivalent g GAE/100 g extract).

Determination of total flavonoid content

al flavonoid content was determined by a colorimetric as- described by Ozsoy et al. (2008). An aliquot of 0.25 ml the extract (1000 μg/ml) was added with 75 μl of $NaNO_2$ w/v) and the mixture was left to stand at room tempera- for 6 min. Thereafter, 150 μl of $AlCl_3$ (10% w/v) was ed and allowed to react for another 5 min before adding 500 μl of NaOH (1 M). The volume was then adjusted to 2.5 ml with distilled water and mixed thoroughly. The absorbance was measured at 510 nm against blank which contained the same mixture without sample. Total flavonoid content was expressed as mg CAE/g extract, through the (+)-catechin calibration curve.

2.8. $ABTS^+$ free radical scavenging assay

The assay was measured based on the ability of antioxidant from the sample to inhibit the 2,2′-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid or ABTS free radical ($ABTS^+$) by comparing with a reference standard (Trolox) (Re et al., 1999). In this assay, $ABTS^+$ radical was generated by reacting ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$) which allowed standing for 15 h in the dark at room temperature. The solution was diluted appropriately with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. To perform the $ABTS^+$ decolorization assay, 200 μl of plant extract (100 μg/ml) was added with 2000 μl of ABTS free radical solution and reacted for 5 min. After that, the absorbance was measured spectrophotometrically at 734 nm against solvent blanks. The Trolox calibration curve ranging from 0 to 200 μM was prepared according to the sample. The percentage of the $ABTS^+$ inhibition was calculated using formula:

Inhibition (%) = (Ac − As)/Ac × 100 where Ac is the absorbance of control; As is the absorbance of sample or standard. Concentration of the extract that inhibit 50% of free radicals (EC_{50} values) were also evaluated.

2.9. Statistical

All results were presented as mean (±SD) values of three replicates. Analysis of variance (ANOVA) was performed using SPSS V. 19 (SPSS Inc., Chicago, IL, USA) and mean values were statistically different at $P \leqslant 0.05$. The significantly different results were further separated using the Tukey multiple range test (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Nutritional compositions

Nutritional compositions of immature and mature corn silk are shown in Table 1. Overall, the moisture content of immature silks (89.31%) was significantly higher than mature silks (84.42%) (P value <0.05). However, no significant difference of the same component was observed for immature (4.15%) and mature (3.90%) corn of the oven-dried silks. The lipid and protein composition of immature silks were significantly higher than those of mature silks (P value <0.05). Lipid content of immature silks was 1.27% and has dropped to 0.66% in the mature silks. Meanwhile, the protein content of immature silks (12.96%) was also higher than its mature counterpart (8.95%) (P < 0.05). There was no significant difference between the ash and carbohydrate compositions of both immature and mature silks (P > 0.05). The ash (5.28%) and carbohydrate content of immature silks (27.80%) were slightly lower than those of the mature silks. The ash and carbohydrate content of mature silks obtained were 5.51% and 29.74%,

Table 1 Nutritional compositions (%, dry basis) of immature and mature corn silk.

Nutritional compound	Corn silks	
	Immature silks	Mature silks
¹Moisture (fresh)	89.31 ± 0.74ᵃ	84.42 ± 0.65ᵇ
²Moisture (oven-dried)	4.15 ± 0.21ᵇ	3.90 ± 0.22ᵇ
³Crude lipid	1.27 ± 0.16ᵃ	0.66 ± 0.17ᵇ
²Crude protein	12.96 ± 0.38ᵃ	8.95 ± 0.21ᵇ
²Ash (%)	5.28 ± 0.13ᵃ	5.51 ± 0.24ᵃ
³Carbohydrate	27.80 ± 2.25ᵃ	29.74 ± 1.26ᵃ
²Total dietary fiber (g/100 g)	48.50 ± 2.88ᵃ	51.24 ± 1.50ᵃ

[a,b]Mean values with different letters in the same row are significantly different ($P < 0.05$).
[1] Moisture content determined by fresh weight.
[2] Dry basis.
[3] Calculated by difference [= 100−(crude lipid + crude protein + TDF + ash + moisture)].

respectively. Regarding the TDF composition, both immature and mature silks displayed high content of TDF comprising of 48.50 g/100 g and 51.24 g/100 g, respectively.

3.2. Mineral composition

Mineral contents (Ca, Mg, K, Na, Cu, Fe, Mn and Zn) of the silks from immature and mature corns were shown in Table 2. In general, the content of major elements such as Ca, Mg, K and Na in immature silks was statistically different from the mature silk. Ca and Mg content were much higher in immature silks amounting 1087 and 1219.17 µg/g, respectively compared to the mature silks which contained 707.04 µg/g of Ca and 361.50 µg/g of Mg. However, the content of K and Na was significantly higher in mature silks compared to those of immature silks. K and Na content of mature silks were 35671.67 and 266.67 µg/g, respectively. Immature silks were found to contain lower amount of K (26281.67 µg/g) and Na (190.67 µg/g).

Both immature and mature silks contained appreciable amounts of trace elements (Table 2). The content of Cu, Fe, Mn and Zn varied from 4.12 to 5.60, 2.17 to 4.50, 32.17 to 35.57 and 35.92 to 46.37 µg/g, respectively. Cu and Zn were found significantly higher in immature silks which were 5.60 and 46.37 µg/g, respectively. For mature silks, the content of these minerals was 4.12 and 35.92 µg/g, respectively. In contrast, Fe content was found to be significantly higher in mature silks (4.50 µg/g) compared to the immature silks (2.17 µg/g). Mature silks also had a slightly higher content of Mn (35.57 µg/g) than the immature ones (32.17 µg/g), but the contents were not statistically different ($P > 0.05$).

Table 2 Mineral contents of immature and mature corn silk.

Minerals level (µg/g)		
Minerals	Immature silk	Mature silk
Macro elements		
Calcium (Ca)	1087.08 ± 105.51ᵃ	707.04 ± 94.41ᵇ
Magnesium (Mg)	1219.17 ± 143.07ᵃ	361.50 ± 20.53ᵇ
Potassium (K)	26281.67 ± 1379.7ᵃ	35671.67 ± 2466ᵇ
Sodium (Na)	190.67 ± 22.61ᵃ	266.67 ± 15.65ᵇ
Minor elements		
Copper (Cu)	5.60 ± 0.4ᵃ	4.12 ± 0.38ᵇ
Iron (Fe)	2.17 ± 0.15ᵃ	4.50 ± 0.49ᵇ
Manganese (Mn)	32.17 ± 3.14ᵃ	35.57 ± 2.26ᵃ
Zinc (Zn)	46.37 ± 4.21ᵃ	35.92 ± 4.24ᵇ

Values are mean ± SD of six replicates.
[a,b]Mean values with different letters in the same row are significantly different ($P < 0.05$).

Table 3 Recovery of extract of silks from immature and mature corns.

Recovery of extract (%)		
Extract	Immature silks (mean + SD)	Mature silks (mean + SD)
Water	14.63 ± 0.36ᵃ	13.69 ± 0.55ᵇ
Ethanol	27.73 ± 0.40ᵃ	3.29 ± 0.27ᵇ
Ethyl acetate	3.27 ± 0.08ᵃ	1.25 ± 0.07ᵇ

[a–b]The different letters in the same row are significantly different ($P < 0.05$).

Table 4 Total polyphenol content of silks from immature and mature corns.

Total polyphenol (mg GAE/g extract)		
Extract	Immature silks (mean + SD)	Mature silks (mean + SD)
Water	35.35 ± 2.17ᵇ	64.22 ± 2.55ᵃ
Ethanol	92.21 ± 3.59ᵃ	49.88 ± 2.87ᵇ
Ethyl acetate	6.70 ± 0.51ᶜ	4.96 ± 0.53ᶜ

[a–c]The different letters in the same row are significantly different ($P < 0.05$).

3.3. Extract recovery, total polyphenol and flavonoid content

The percentage of recovery, total phenolic and flavonoid contents of immature and mature silks using water, ethanol and ethyl acetate solvent are shown in Table 3. Apparently, the water, ethanol and ethyl acetate extracts of immature silks exhibited significantly higher percentage of recovery ($P < 0.05$) compared to the mature silks. The highest recovery of immature silks was recorded in ethanol (27.73%) extract followed by water extract (14.63%). Conversely, mature silk displayed the highest percentage of recovery in water extract (13.69%) compared to ethanol extract (3.29%). Both immature and mature silks extracted with ethyl acetate recorded the lowest percentage of recovery that was 3.27% and 1.25%, respectively.

The immature and mature silks had showed significant differences in total polyphenol and flavonoid content ($P < 0.05$). Total polyphenol content of crude extracts of immature and mature silks varied from 6.70–92.21 mg GAE/g extract to 4.96–64.22 mg GAE/g extract, respectively (Table 4). As for immature silks, the ethanol extract displayed the highest polyphenol (92.21 mg GAE/g extract) content followed by the water (35.35 mg GAE/g extract) and ethyl acetate (6.70 mg GAE/g extract) extract. In contrast, the highest total polyphenol content in mature silk was displayed by water 'able 5 Total flavonoid content of silks from immature and ıature corns.

otal flavonoid (mg CAE/g extract)		
xtract	Immature silks (mean + SD)	Mature silks (mean + SD)
Vater	8.40 ± 0.48[a]	2.31 ± 0.12[b]
thanol	7.55 ± 0.37[a]	1.96 ± 0.20[b]
thyl acetate	0.66 ± 0.02[b]	2.10 ± 0.19[a]

-b)The different letters in the same row are significantly different ᴾ < 0.05).

'able 6 EC_{50} values of immature and mature silk extracts.

	EC_{50} value (mg/ml)		
	Ethanol extract	Water extract	Ethyl acetate extract
nmature silk	0.478 ± 0.030[c]	0.751 ± 0.240[b]	2.870 ± 0.110[a]
Iature silk	0.799 ± 0.100[c]	1.489 ± 0.166[b]	6.290 ± 0.830[a]
'rolox	0.038 ± 0.005		

-c)Different letters in the same row are significantly different P < 0.05).

tract (64.22 mg GAE/g extract) followed by the ethanol).88 mg GAE/g extract) and ethyl acetate (4.96 mg GAE/g tract) extract.

As for total flavonoid content, the immature silks exhibited gher content compared to the mature silks (Table 5). In addi- n, the water extract of immature and mature silks gave the ghest flavonoid content that was 8.40 mg CAE/g extract and 31 mg CAE/g extract, respectively compared to other tracting solvents employed. Meanwhile, the ethanol and hyl acetate extract of immature and mature silks showed wer content of flavonoid. The flavonoid content of ethanol tract of the immature silk was 7.55 mg CAE/g extract and gnificantly higher than that of the mature silk 96 mg CAE/g extract). For ethyl acetate extract, the flavo- ids obtained from mature silks (2.10 mg CAE/g) were higher mpared to the immature silk (0.66 mg CAE/g extract) (see ıble 6).

4. ABTS free radical scavenging capacity

ıe antioxidant capacity of immature and mature silk extracts measured by using the $ABTS^+$ free radical scavenging ethod is shown in Fig. 1(a–c). Overall, all extracts had owed a dose-dependent response when exposed to different ncentration of extracts (0.1–1.6 mg/ml). Higher percentage inhibition was observed at higher concentration. Appar- tly, the water, ethanol and ethyl acetate extract of immature k demonstrated a higher percentage of inhibition compared the same extracts of mature silk. Among all three extracts, e ethanol extract of both immature and mature silks exhib- d the highest scavenging capacity followed by the water ıd ethyl acetate extract. These results indicated that antioxi- ınt compounds present in immature silk extracts are prone to onate hydrogen atom to ABTS free radical giving rise to its scavenging capacity. The percentage of inhibition of ethanol extract was ranged from 23.50% to 78.40% in immature silk and 17.40% to 67.40% in mature silk (EC_{50} value of 0.478 mg/ml and 0.799 mg/ml, respectively) (Table 4). A lower scavenging capacity was observed in water and ethyl acetate extract of both immature and mature silks. The percentage of inhibition of immature silk water extract (19–57%) was however slightly higher than that of mature silk (15.44–53.5%) recording the EC_{50} value of 0.751 mg/ml and 1.489 mg/ml, respectively. The lowest antioxidant capacity of both silks was exhibited by ethyl acetate extract. The ethyl acetate extract of immature silk however showed lower EC_{50} value (2.87 mg/ml) compared to the mature silk (6.29 mg/ml). The inhibition of immature and mature silks was ranged from 13.4% to 35% and 15.56% to 44.36%, respectively.

4. Discussion

4.1. Nutritional compositions

In moisture content, the oven-dried silks which were dried at 55 °C for 48 h recorded the moisture content of 3.90–4.15%. The differences in moisture content of oven-dried samples in our study with other samples may be due to the differences in the temperature used and duration of drying. The moisture content obtained here was lower than the dried pericarp and seeds of bitter melon (5.0–13.4%) of different maturity stages which had been dried at 40 °C for only 24 h (Horax et al., 2010). The moisture content of both immature and mature silks however was found higher than some dried apple and orange residues (2–4%) (Figuerola et al., 2005), but lower than mango peel powder (10.5%) (Ajila et al., 2008) and its seed (8.5%) (Abdalla et al., 2007).

Among other plant byproducts, the lipid content of maize silks was lower than tomato peel (Elbadrawy and Sello, in press) but comparable with some plant pods (Mateos-Aparicio et al., 2010). Maize silk cuticle was composed of hydrocarbons of silk waxes that was involved in certain hydrocarbon biosynthesis (Perera et al., 2010). Therefore, the accumulation of hydrocarbons in different maturity stage may influence lipid composition.

Immature silk was obtained from unpollinated cob while the mature silk was taken from fully ripen and developed corn fruit. The protein content of immature and mature silks may be influenced by the functions and biosynthesis of amino acids taking place during developmental processes of pollinated and unpollinated silk tissue. It has been reported that amino acids were actively metabolized in immature cob at early stage of silk emergence in order to regulate the seed growth (Seebauer et al., 2004). The protein content of both immature and mature maize silks in the present study was slightly higher than some vegetables (2–4%) (Odhav et al., 2007) and underutilized plant parts such as mango peels (3.6%) and pods (Mateos-Aparicio et al., 2010).

The TDF content of maize silks was higher than some other underutilized plant parts such as seed (Hainida et al., 2008) peel (Elbadrawy and Sello, 2011), and brans (Abdul-Hamid and Luan, 2000). Therefore, maize silks could be considered as a rich source of dietary fiber thus may confer positive health benefits.

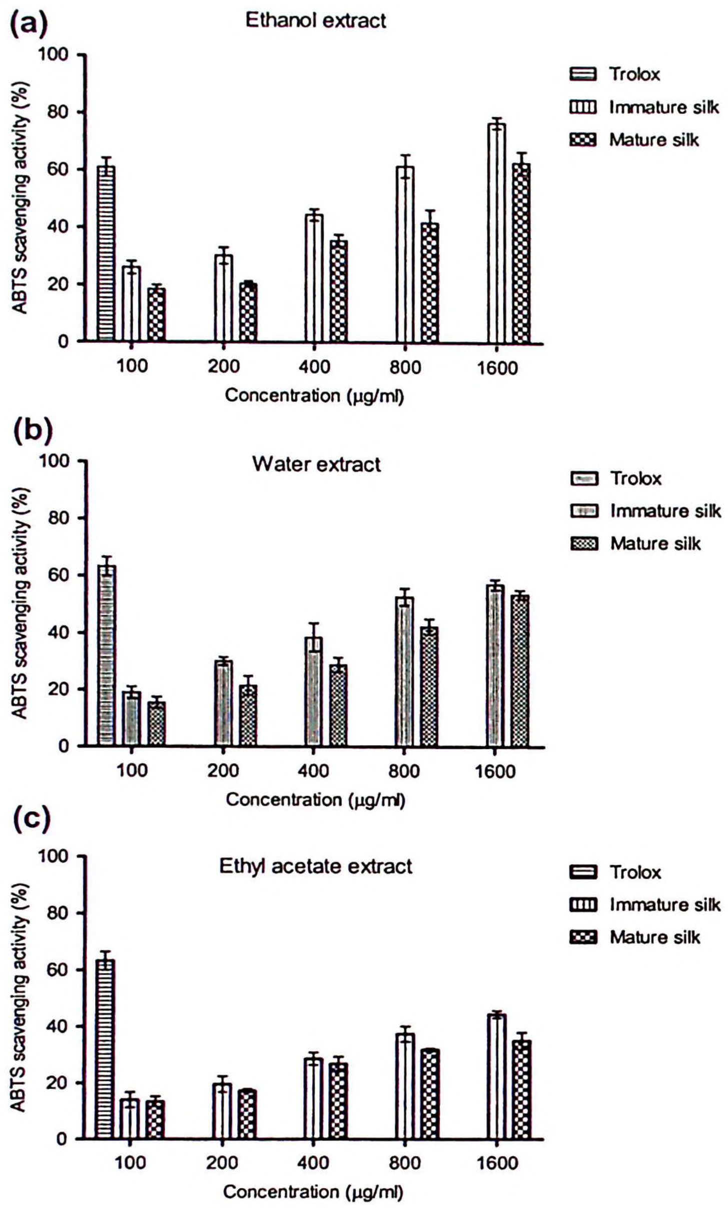

Figure 1 (a–c): ABTS scavenging capacity of ethanol, water and ethyl acetate extract of immature and mature silks.

4.2. Mineral content

Mineral composition of the silks immature and mature parts was varied. The variations could be attributable to the difference cultivar, plant nutrition, climate and soil conditions (Hamurcu et al., 2010). The distribution of mineral composition is related to certain functions of the plant part during development. Ca is one of essential mineral which is required for structuring of cell wall and membranes (Evans et al., 2001). K ion is very important in biophysical and biochemical functions as it is highly mobile in plants (Szczerba et al., 2009). In stigma, K ion involves in regulation growth of a pollen tube (Holdaway-Clarke and Hepler, 2003; Zienkiewicz et al., 2011). Zn is required in protein synthesis, genetic entities stability and metabolism of carbohydrate and lipid. Mn plays the role as enzymes activator in tricarboxylic acid cycle (Xue et al., 2004), photosynthetic function (Lidon et al., 2004) photosynthesis, fatty acid and carotenoid synthesis (Santandrea et al., 2000). Mg is abundant in plant tissue and is associated with a wide range of cellular functions including photosynthesis (Gardner, 2003), protein synthesis which involves in ribosomal structures and functions (Maathuis, 2009).

4.3. Extract recovery, total polyphenol and flavonoid content

These results demonstrated clearly that the choice of varying polarities solvent had influence on the extractability of bioactive compounds (Trabelsi et al., 2010). The nature of solvent ay explain on its capacity to recover as many antioxidants as ıssible and solvent having higher polarity exerted higher per-ntage of recovery. In addition, the different affinities of the traction solvent such as extraction conditions, solvent polar-and temperature toward chemical constituents in plant aterials could attribute to the extract recoveries (Moure al., 2001). In other study, some exotic fruits showed higher eld of polyphenol when combination of ethanol with other lvent was used (Martinez et al., 2012). Successive isolation flavonoid compounds from tea leaves (*Camellia sinensis*) ıs reported by using ethanol and water solvents (Yang al., 2009). Maize silks extracted with ethyl acetate had little coveries and found to be lower than the same solvent used to tract tea leaves (Farhoosh et al., 2007).

Maturity stage may influence accumulation of bioactive mpounds. In such case, older plant having lower extract covery than the immature ones had has also been observed tea leaves extracts (Farhoosh et al., 2007). In apple for in-ance, the accumulation of total flavonoid and chlorogenic id was higher in immature fruit but the level had decreased the fruit reaches the mature and ripening stage (Awad al., 2001). Some other reasons are due to the physiological ıd structural changes taking place during the growth period ıd fertilization requirements (Salvador et al., 2007).

4. ABTS free radical scavenging capacity

this assay, the antioxidant from extract acts as hydrogen do-ırs to $ABTS^+$ free radical thus inhibits oxidation process. ılyphenols include many classes of compounds (phenolic ids, flavanols, xanthones, kaempferol etc.) and act as free dical scavengers. Flavonoid which is the major class of poly-ıenols exhibits strong antioxidant capacities (Fraga and Ote-ı, 2011). However, the antioxidant capacity has decreased ıring maturity stage and this may be associated with appar-ıt decrease in the total polyhenols and flavonoid contents as ımpared to the immature part. As the plant grew older, new osynthesis of polyphenols may interrupt or end thus decrease e content of polyphenols. The reduction of polyphenol con-ıt has also been attributed by oxidation of polyphenols dur-g the maturity stage (Shwartz et al., 2009).

Conclusions

ı conclusion, both immature and mature silks could be con-dered as good source of nutritional compositions and antiox-ant capacity. The immature silks contained significantly gher content of moisture ($P < 0.05$) than the mature silks. ipid and protein content decreased nearly 50% and 31%, spectively in mature silks compared to those in immature lks. The composition of ash, carbohydrate and total dietary ber of both silks was not significantly different ($P > 0.05$). nmature silks contained significantly higher level of Ca, g, Cu and Zn while other minerals such as K, Na and Fe ere significantly higher in the mature silks ($P < 0.05$). Imma-re silks had significantly higher content of polyphenol and ıvonoid content ($P < 0.05$) than the mature silk which was ıown by the ethanol and water extract, respectively. Both eth-ıol extracts of immature and mature silks possessed strong e radical scavenging capacity compared to the water and hyl acetate extract. The ethanol extract of immature silk however had the highest antioxidant capacity compared to same extracts of mature silks.

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Aqueous Extract of Cornsilk Confers Mild Diuretic Activity in Normal Rats

(Ekstrak Akues Sutera Jagung Cetus Aktiviti Diuretik Sederhana dalam Tikus Normal)

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ABSTRACT

Cornsilk is traditionally used to treat illnesses related to kidney and as diuretic agent. The study was performed to evaluate the effectiveness of Malaysian cornsilk in elevating diuresis and their dose response relationship in normal Sprague-Dawley *rat. The diuresis activity was determined by administered the rats with different dose treatments of 400, 500, 600, 700 and 800 mg/kg. Cumulative urine was significantly increased with the dosage levels (400-600 mg/kg) ranging from 14.06 - 20.13 mL. Cumulative urine of aqueous extract of cornsilk (AEC) at 400 mg/kg (14.06 mL) and 500 mg/kg (15.21 mL) treatments found to be significantly lower than positive control (21.25 mL). In addition, Na^+ content was significantly higher compared with negative control at dosages of 500, 600, 700 and 800 mg/kg. At any rate, K^+ and Cl^- content of all AEC treatments were not significantly different during 24 h monitoring. The pH values were increased paralleled with the increment of AEC dosages, though it was not significant. On the other result, the ED_{50} of AEC was observed at 454.10 mg/kg. Malaysian AEC had shown a mild diuretic activity in elevating urine and Na^+ content at dosages from 500 to 800 mg/kg. Whilst, AEC also showed an effect of potassium sparing diuretics. Thus, it is suggested that Malaysian cornsilk can be used as an alternative natural diuretic agent.*

Keywords: Aqueous extract; cornsilk; diuretic property

ABSTRAK

Sutera jagung kerap kali digunakan secara tradisi untuk merawat masalah kesihatan yang berkaitan dengan buah pinggang dan sebagai agen diuretik. Kajian telah dilakukan untuk menilai keberkesanan sutera jagung Malaysia dalam meningkatkan diuresis dan perkaitan respons dos dalam tikus Sprague-Dawley *normal. Aktiviti diuresis telah ditentukan dengan memberi rawatan kepada tikus pada dos yang berbeza (7 kumpulan/6 ekor setiap kumpulan) iaitu 400, 500, 600, 700 dan 800 mg/kg. Air suling digunakan sebagai kawalan negatif manakala hidroklorothiazid pula sebagai kawalan positif. Keputusan kajian menunjukkan urin kumulatif meningkat secara signifikan dengan peningkatan dos (400-600 mg/kg) dalam julat daripada 14.06-20.13 mL. Urin kumulatif ekstrak akues sutera jagung (AEC) pada dos 400 (14.06 mL) dan 500 mg/kg (15.21 mL) didapati lebih rendah daripada kawalan positif (21.25 mL). Sebagai tambahan, kandungan Na^+ adalah lebih tinggi secara signifikan berbanding kawalan negatif pada dos 500, 600, 700 dan 800 mg/kg. Pada semua kadar, kandungan K^+ dan Cl^- bagi semua perlakuan AEC adalah tidak signifikan dalam masa 24 jam pemerhatian. Nilai pH meningkat selari dengan peningkatan dos AEC, walaupun tidak signifikan. Dalam keputusan lain,* ED_{50} *AEC adalah sebanyak 454.10 mg/kg. AEC Malaysia telah menunjukkan aktiviti diuretik sederhana dalam meningkatkan kandungan urin dan Na^+ pada dos 500 hingga 800 mg/kg. Sementara itu, AEC juga menunjukkan kesan penahanan diuretik kalium. Oleh itu, sutera jagung Malaysia dicadangkan boleh digunakan sebagai agen diuretik semula jadi.*

Kata kunci: Ekstrak akues; sifat diuretik; sutera jagung

INTRODUCTION

Natural plant has been used for centuries as a folk medicine to treat various illnesses. Many people presume natural plant safer than normal drug used to treat certain diseases. This situation has triggers scientist interest in investigating further the benefits of natural plant as an alternative remedies. Corn is one of the food item commonly consumed in Malaysia. Unfortunately, by-product of corn cob namely cornsilk which is popular as a traditional medicine amongst North Americans, Chinese and Indians is discarded upon harvesting of corn. Therefore, an effort is taken to investigate the possibility of Malaysian cornsilk as a therapeutic agent to treat diuresis.

Cornsilk is from *Zea mays* (Gramineae) family. It is found inside the husk and hardly reveals until the emergence of yellow pale silk at the end of the husk. Cornsilk has been reported to have antioxidant properties (Ebrahimzadeh et al. 2008; El-Ghorab et al. 2007; Eman 2011; Liu et al. 2011), antiprostatitis and antispasmodic (Buhner 2007). In addition, cornsilk is well known to treat infection and cystitis (Steenkamp 2003), help pass stone from kidney and others related to renal illness (Maksimovic et al. 2005). Cornsilk was reported to contain sitosterol, stigmasterol, fatty and volatile oil, saponin, glucoside substance and vitamin C and K (Bobryshev 1962). In previous study, cornsilk was also reported to consist of saponin, terpenoid, glycoside, alkaloid, tannin and phlobatannin, flavonoids and phenol.

Herbs are common ingredient used as food flavouring over hundreds of years and generally regarded as natural remedies for certain particular diseases. Herbs and botanicals interest as a health benefits amongst consumers is on the rise (Foote & Coohen 1998). In recent years, food manufacturers have focused their products with health benefits using herbs or botanicals as their key ingredients. In the food industries, it is commonly known as functional food or products which have an add-on value to the products. However, approximately 80% of world's population consistently rely on plants for health promote and remedial (Esiyok et al. 2004). Instead of relying on a modern treatment, peoples are harmonizing natural products to gain a complete recuperation.

Since cornsilk is popular in treated diseases related to renal, it leads us to investigate the diuretic activity of Malaysian's cornsilk. Even though many researchers have been done on the diuretic activity of cornsilk, but there were different variations conclusion observed among researchers. Habitually, there is variability of phytochemicals amongst plants namely genotype, size and maturity, soil conditions, fertilization, irrigation, pesticide utilization, disease and pests, location and climate and season (Xin et al. 2006). Furthermore, cornsilk is rarely known used as traditional medicines among Malaysian population. Therefore, the aimed of the present study was to evaluate the diuretic activity of Malaysian cornsilk and the dose response relationship of its aqueous extract.

MATERIALS AND METHODS

DRUGS

Hydrochlorothiazide is supplied by the Department of Pharmacy of Hospital Universiti Sains Malaysia. This drug is used as reference diuretic agents.

PLANT MATERIAL

Corn fruit was bought from Pasar Siti Khatijah wet market located in Kota Bharu town, Kelantan, Malaysia. Cornsilk was then detached from corn fruit and the inside tassel was collected. Cornsilk was dried at 55°C in air oven overnight until golden yellowish colours of cornsilk were achieved. Dried cornsilk was ground and formed into powder used domestic blender (National; MX-895).

PREPARATION OF EXTRACTS

Aqueous extract was prepared by boiling 80 g of cornsilk powder with distilled water for 30 min. The ratio of cornsilk to distilled water used was 1:15 (w/v). The solution was then filtered through Advantec filter paper (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 it completely concentrated. This concentrated extract was used to prepare different dosages of aqueous extract of cornsilk (AEC) at 400, 500, 600 and 700 mg/kg.

EXPERIMENTAL ANIMALS

Sprague Dawley (SD) male adults weight ranged between 250-300 g were used in the experiments and treated according to the ethical guideline set by animal ethic committee of Universiti Sains Malaysia (USM/Animal Ethics Approval/2009/(48)(153)). The animals were grouped in the cage of five, with 12 h light dark cycle and placed in Animal Research and Service Centre (ARASC) of USM. The animal was fed with a balanced pellet diet and tap water was administered *ad libitum*. Prior to the start of experiment all animals was placed in metabolic cage individually and fasted overnight with free access to water. Total of 25 mL/kg body weight (BW) of aqueous extract of cornsilk (AEC) with different dosages of 400, 500, 600 and 700 mg/kg were given orally to each rat as shown in Table 1. Each solution was prepared freshly prior to administration. The care and handling of rats were in accordance of standard guidelines for laboratory animal use and care.

ELECTROLYTE CONTENTS IN AEC

An aqueous extract were dissolved in distilled water. The contents of sodium, potassium and chloride in mmol/L were measured using ion photometer.

DIURETIC STUDY

The modified method of Arafat et al. (2008) was used to determine the diuretic effect. The morning after have been fasted overnight, the rats were given orally, 25 mL/kg of treatment solution and then were placed in each metabolic cage. Urine excreted for the next 24 h were collected. The volume and pH of each rat were measured and then kept under -18°C prior to other analysis. Electrolyte content of sodium, potassium and chloride in AEC were determined by ion selective electrodes (Architect C8000 Analyzer). Osmolality of urine were determined by osmometer (Osmomat, 030).

REPEATED ADMINISTRATION OF AEC

The rats were administered with the same dose of AEC as day 1 for another 4 days. The similar parameters as day 1 are observed.

STATISTICAL ANALYSIS

All results were presented as mean±S.E.M (standard error of means). Statistical significant differences between groups were evaluated by one-way analysis of variance (ANOVA). The p value less than 0.05 were used to represent significant differences.

TABLE 1. Effect of diuretic activity produced by different treatment groups

Treatment	Cumulative urine volume (mL/24 h)	Diuretic index[a]	Diuretic activity[b]	pH	Osmolality
Distilled water	14.94 ± 0.27⁺	1.00	0.70	8.24 ± 0.08	1598.50 ± 73.32
Chlorotiazide (10 mg/kg)	21.25 ± 1.14*	1.42	1.00	8.28 ± 0.22	1254.00 ± 111.93
400 mg/kg AEC	14.06 ± 0.37⁺	0.94	0.66	8.20 ± 0.12	1568.00 ± 8.00
500 mg/kg AEC	15.21 ± 0.96⁺	1.09	0.76	8.31 ± 0.20	1488.00 ± 149.95
600 mg/kg AEC	20.13 ± 0.61*	1.35	0.94	8.41 ± 0.24	1456.88 ±77.29
700 mg/kg AEC	19.63 ± 0.53*	1.31	0.92	8.81 ± 0.19	1509.13 ± 45.10
800 mg/kh AEC	20.00 ± 0.58*	1.34	0.94	8.33 ± 0.17	1548.00 ± 45.54

The results showed the mean values and standard errors of measurements (n=8) in each groups. One-way ANOVA was applied followed by post hoc comparison. The differences in each column show as; * $p<0.05$ with respect to distilled water.
⁺$p<0.05$ with respect to chlorothiazide (positive control). [a]Diuretic index = (cumulative urine volume of treated group)/ (cumulative urine volume of control group).
[b]Diuretic activity = diuretic index of treated test extract/diuretic index of standard drug

RESULTS

PLANT EXTRACT

AEC yield obtained was 40.8%. There was a report on phytochemicals found in cornsilk contains of flavonoids, saponin, tannins, phlobatannins, phenols, alkaloids and cardiac glycosides (Solihah & Wan Rosli 2012).

ELECTROLYTE CONTENTS IN AEC

The amount of sodium, potassium and chloride in 10 mg/mL of AEC are illustrated in Figure 1. The sodium level found was 20 mmol/L. Meanwhile the potassium and chloride content were 28 and 16 mmol/L, respectively.

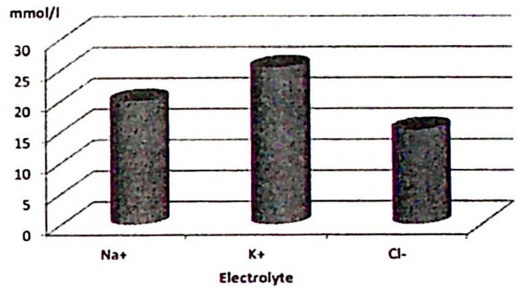

FIGURE 1. Electrolyte content of AEC in 10 mg/mL

VOLUME, PH AND OSMOLALITY OF URINE EXCRETED

The cumulative urine collected during 24 h showed significant differences between AEC treatments to distilled water and chlorothiazide group (Table 2). The urine excretion that received AEC doses at 600, 700 and 800 mg/kg were significantly higher compared to distilled water. Meanwhile in comparison to chlorothiazide, AEC dose at 400 and 500 mg/kg seemed to be statistically lower. Therefore, the urine amount of AEC dosage at 600 and 700 were not statistically significant to chlorothiazide. Immensely doses of 600, 700 and 800 mg/kg are equally effective to excrete urine at same rate with 10 mg/kg of chlorothiazide. Above of all, the maximum urine excreted of AEC dose monitored at 600 mg/kg and became faintly stagnant at dose of 700 and 800 mg/kg. Diuretic index of AEC ranged from 0.94 to 1.35, meanwhile diuretic activity ranged from 0.66 to 0.94 (Table 2). Diuretic activity is a good indicator for the efficacy. From the table, AEC doses at 600, 700 to 800 mg/kg (0.94, 0.92 and 0.94) have a good efficacy for diuretic activity when compared to hydrochlorothiazide (1.00). Other than that, the urinary pH showed no significant difference with respect to distilled water. However, urinary pH for AEC treatment at doses 600 and 700 mg/kg were slightly higher from distilled water.

TABLE 2. The electrolytes content of urine after 24 h administered with AEC

	Na^+	K^+	Cl^-	Saliuretic index[a]			Na^+/Cl^-
				Na^+	K^+	Cl^-	
Distilled water	40.63 ± 4.12⁺	246.25 ± 10.79	86.38 ± 2.79	1.00	1.00	1.00	0.47
Chlorotiazide (10 mg/kg)	90.38 ± 4.16*	243.75 ± 7.91	104.62 ± 4.20	2.22	0.99	1.21	0.86
400 mg/kg AEC	52.13 ± 2.49⁺	258.38 ± 5.64	89.38 ± 6.72	1.28	1.05	1.03	0.58
500 mg/kg AEC	62.75 ± 3.60*⁺	255.00 ± 8.28	110.12 ± 8.66	1.60	1.04	1.27	0.57
600 mg/kg AEC	63.50 ± 1.79*⁺	224.12 ± 10.69	83.38 ± 4.78	1.56	0.91	0.97	0.76
700 mg/kg AEC	58.67 ± 3.74*⁺	232.87 ± 5.46	79.50 ± 6.16	1.24	0.95	0.92	0.63
800 mg/kg AEC	62.33 ± 3.28*⁺	236.60 ± 18.75	107.7 ± 8.29	1.53	0.96	1.25	0.58

The results showed the mean values and standard errors; (n=8) in each groups. Statistical analyses used are one-way ANOVA followed by post hoc comparison. The differences in each column show as; * $p<0.05$ with respect to distilled water and ⁺$p<0.05$ with respect to chlorothiazide.
[a]Saliuretic index = problem group (mmol/L)/control group (mmol/L)

EFFECT ON URINARY ELECTROLYTE EXCRETION

The urinary Na^+ excretion found among AEC doses are significantly higher compared with distilled water (40.63 mmol/L), except AEC at dose of 400 mg/kg (52.13 mmol/L). However, all AEC doses ranging from 400 to 800 mg/kg are significantly lower when compared with hydrochlorothiazide (90.38 mmol/L). On the other part, K^+ excretions of all AEC doses are not significantly differed from distilled water or hydrochlorothiazide except during dose of 600 and 700 mg/kg which are significantly lower compared with hydrochlorothiazide. Furthermore, Cl^- excretion found no statistical different between AEC group and both control groups. The saliuretic index of Na+ is higher in all extracts compared with control group. Meanwhile, K+ and Cl- found to be lower in all extract than the control group. On the other part, all AEC doses have an increased of Na^+/Cl^- ratio when comparison made to control group. Nevertheless, all ratios are lower than chlorothiazide (0.86) treatment.

MEDIAN EFFECTIVE DOSE OF AEC DIURESIS

During 24 h observation, the AEC showed a significant dose dependant diuretic activity in terms of cumulative urine excreted (Figure 2). The present study indicated that there is no significant increased at doses of 100, 200 and 300 mg/kg of AEC (unpublished data) and so at a dose of 400 mg/kg (14.06±0.37 mL). The effect of diuresis started to increase at a dose of 500 mg/kg (15.21±0.96 mL) and 600 mg/kg (20.13±0.61 mL). Later, it becomes plateau at dose of 700 mg/kg (19.63±0.53 mL) and 800 (20.00±0.58 mL). From this observation, the AEC shows a pharmacological effect of median effective dose (pED_{50}) at 454.10 mg/kg, as illustrated in Figure 2. The maximum effect of the diuresis was observed at 20.93±6.65 mL, while the baseline at 11.02±5.26 mL.

EFFECT OF REPEATED ADMINISTRATION OF AEC ON URINE EXCRETED

A repeated exposure of AEC at dose of 400, 500, 600, 700 and 800 mg/kg during 5 consecutive days showed no statistical difference in cumulative urine, pH, osmolality, sodium, potassium and chloride when compared with day 1 of respective group (Figure 3).

DISCUSSION

Diuretics are synthetic drug which is used to increase the volume of urine excreted from body. This is the first study to demonstrate the diuretic activity of Malaysian cornsilk. Apart from that, Wright et al. (2007) has wind up about 19 plants to show the potential diuretic effects. Diuretics have a wide definition and commonly referred as excretion of water and Na^+ in larger volume (Rang et al. 2007). Whereas diuresis commonly known as a process to increase the formation of urine. In the present study, AEC was seemed to be able to present as mild diuretics as it is capable to increase urine and Na^+ excretion. The electrolytes content in the AEC extract showed that Na^+ (20.0 mmol/L) has lower value compared with K^+ (28.0 mmol/L), while Cl- is the lowest with 16.0 mmol/L in every 10 mg/mL. These results are lower compared to *Erica multiflora* and *Cyanadon dactylon* which have been reported by Sadki et al. (2010). *Erica multiflora* contained 33.9 and 25.8 mmol/L of Na^+ and K^+ while *Cyanodon dactylon* contained 75.26 and 56.77 mmol/L of Na^+ and K^+, that was much higher compared with *Erica multiflora*. The electrolytes content of both plants have been converted into mmol/L, so that relative comparison can be done.

On the other part, AEC treatment did not have significant differences among all electrolyte excretion except for sodium content at doses of 500 and 600 mg/kg in comparison to control group (40.63±4.12 mmol/L). In

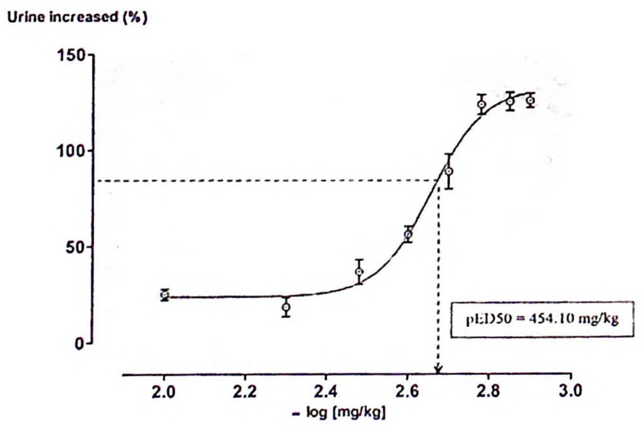

FIGURE 2. Log concentration-dose response curve of urine increased (n=6) generated using Graphpad Prism. Volume at baseline was 11.02±5.26 mL

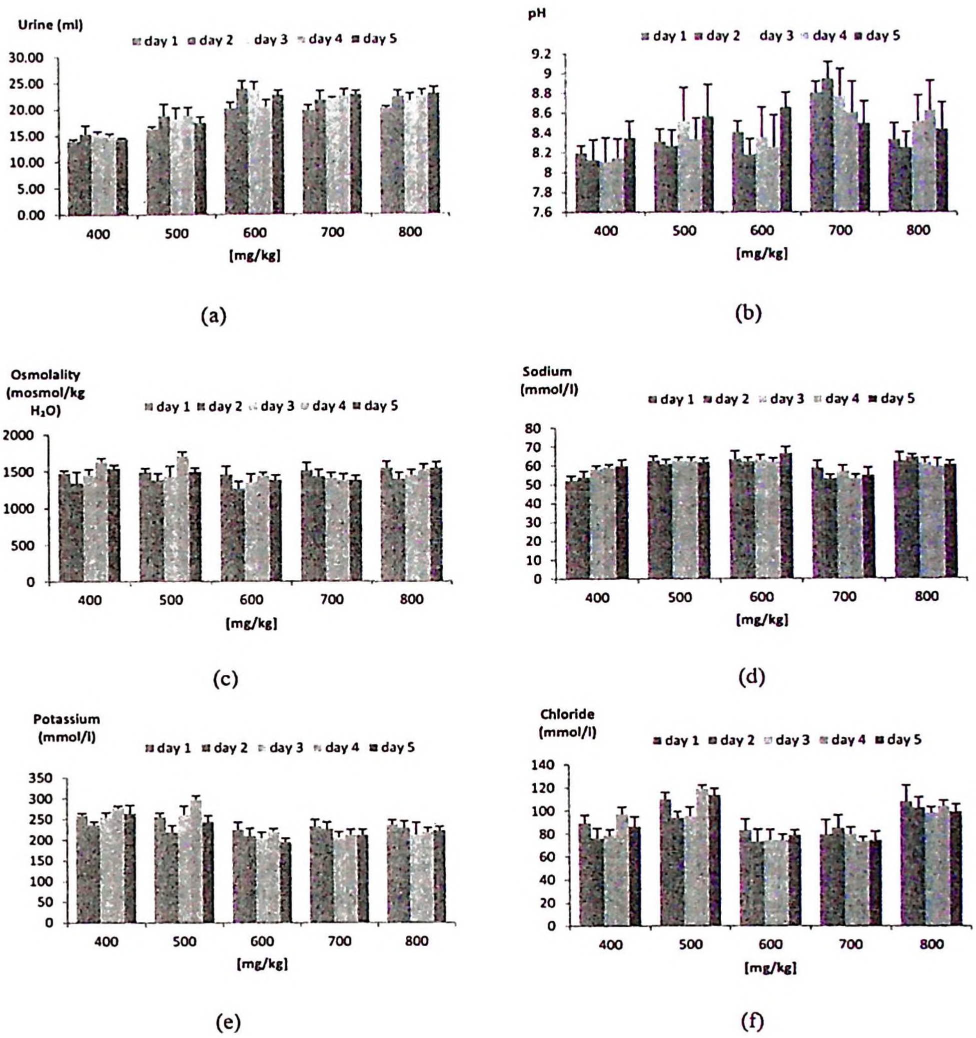

FIGURE 3. Effect of repeated oral administration of CAE on urine excretion (a), pH (b), osmolality (c), sodium (d), potassium (e) and chloride (f). Day of treatment was subjected to 5 days. The urine samples were collected daily. Each bar represents the mean of 6 animals and vertical lines indicated the S.E.M. Asterisks denote the level of significant in comparison to day 1 using one-way ANOVA test, followed by Dunnet T^3 post-hocs test ($p \leq 0.05$)

contrary, Ali et al. (2003) have reported that cornsilk did not show any diuretic action when given alone but yet it did increase sodium, potassium and chloride contents in the urine excreted. These report seemed to be opposite to the present result discovered. Opportunely, Maksimovic et al. (2004) have reported the urine excretion resulted from cornsilk administration to rats. Compared to other studies by Arafat et al. (2008) and Nedi et al. (2004), potassium level in all groups was enormously high. It was reported that normal urine electrolytes excreted during 24 h for Na^+ and K^+ are 200 and 150 mmol/l, respectively (Johnson 2007). However it has also been mentioned, there will be distinction of contents due to diverse strain of animal, supplier, feed and housing condition used. Hence, the atypical value of K^+ excretion in this study may be due to the diet given to the animal.

Interestingly, the insignificant K^+ value found between AEC and distilled water indicates that AEC has demonstrated a possible effect as potassium sparing diuretics. Since, AEC did not alter the K^+ exretion in the urine while elevated diuresis. However, a detailed study has to be performed by *in vivo* test to confirm the potassium diuretics effect (Alarcon-Alonso et al. 2012). In addition, AEC also do not caused over stimulation of renin angiotensin aldosterone system (RAAS) as happened with acute administration of furosemide due to the associated kaliuresis (Cataliotti et al. 2004). The increased of saliuretic index for Na^+ can be observed in all AEC though it was not significant. But the increment was obviously observed at doses of 500, 600 and 800 mg/kg. This result possibly indicated that the inhibition of Na^+ resorption in nephron occured and causes the diuresis (Ratnasooriya et al. 2009). On the other part, the NA^+/Cl^- ratio of AEC doses at 600 and 700 mg/kg showed marked increased. But this result do not give final conclusion for AEC to act like hydrochlorothiazide diuretics as the Na^+/Cl^- ratio do not increased in line with the increased of AEC doses.

Though the pH value among doses did not have significant differences, the trend of alkalization can be observed as the doses increased. Concurrence to Fjellstedt et al. (2001), alkalization of urine enhanced the solubility of cystine, as well as neutralising the acid level in the urine. The present result also showed no alteration of osmolality level. This indicates that, AEC did not alter the impaired basal secretion of ADH (anti diuretic hormone) nor lessened the sensitivity of uniferous tubule that influences the ADH secretion (Osario & Teitelbaum 2002).

Likely, the thiazide diuretics inhibit the Na^+/Cl^- symporter in the distal convulated tubule by competing the Cl^- site and increasing the excretion of Na^+ and Cl^- (Rang et al. 2007). Meanwhile, loop diuretics elevates urine excretion and Na^+ by inhibiting $Na^+/K^+/2Cl^-$ symporter in the thick ascending limb of the loop of Henle (Jackson 1996). Loop diuretics like furosemide increased the excretion of Na^+, K^+ and Cl^-. Nevertheless there are many ways for diuresis to occur. Likewise, herbs that contain of high sodium or potassium (Hook et al. 1993) helps to promote diuresis by inhibit the reabsorption of renal tubular. In contrast to the present study, sodium content of AEC is very low but somehow it significantly increased urine excretion at dose 600 to 800 mg/kg.

The other diuresis mechanism is by arousing the thirst centre in the hypothalamus to boost the fluid intake (Neuman 2002). Besides that, inhibition of ATPase activity can impact the Na^+/K^+ concentration in the epithelial cells of nephron tubular segment which enhance the diuretic activity (Mezesova et al. 2010). The Na^+/K^+ ratio of AEC do have a dose dependant manner but it was not significant. Moreover, particular phytochemicals act as vasoconstrictors agent and aid the release of renal prostaglandins (Gasparotto Junior et al. 2009). When vasoconstrictors are released, prostaglandins amend their effects on kidney by causing compensatory vasodilatation (Rang et al. 2007).

In the previous study, a various phytochemicals was identified in cornsilk (Solihah & Wan Rosli 2012). The component found were saponin, phlobatannins, tannin, flavonoid, phenols, alkaloid and glycosides. These components possibly provoked the diuresis. There are various plants exhibited diuretic activity due to its components. The ursolic acid from wild African olive leaves has demonstrated diuretic activity. Freitas et al. (2011) has suggested this compound also induced diuresis in *Palicourea coriacea*. Moreover, catechin a flavanoid group has been reported to promote urine excretion in human (Donovan et al. 2002). Meanwhile quercetin influenced the diuretic activity of *Hibiscus sabdariffa* (Alarcon-Alonso et al. 2012).

Apart from that, phlobatannins found in *Cnidoscolus aconitifolius* has been suggested to be responsible for the diuretic action of the plant (Awoyinka 2007). Hence, there are many compounds contained in crude AEC which could possibly provoke diuresis. It is possible that it works individually or synergistically with flavonoids, saponin or organic acid (Sadki et al. 2010). At this point, there is no scientific evidence associated the active compounds responsible for diuretic activity in cornsilk. Therefore, further study need to be done to examine the particular compound which responsible to this activity.

Since we do not examine the explicitly mechanism of the diuresis in this study, we can only suggest the possible mechanism happened during diuresis of AEC. The diuretic action of AEC probably was implicated by arousing the thirst centre of hypothalamus due to the action of compound presence in the AEC. These compounds probably act individually or synergistically. This mechanism probably has influenced the water intake of those rats treated with AEC doses, since all rats have access to water during this investigation. Another mechanism might involve with this diuretic activity is the action of AEC as potassium sparing diuretics. However, detail study needs to be performed in order to investigate the particular mechanism.

Another observation is evaluated during the study that is specifically on the effective dose of AEC (Figure 2). The plateau effect of the extract may be to the effect of different features of compound action or due to the toxic effect which was noticeable at higher doses (Sangma et al. 2010). This trend most probably related to the various compounds composed in the extract, since there maybe intervention among compound as the doses of extract increased (Ncdi et al. 2004). The pharmacological effective dose of diuresis study was unavailable. Therefore, a comprehensive comparison is unable to be discussed.

During repeated oral administration of AEC over 5 days, there are no statistical differences found in all parameters studied including cumulative urine, pH, osmolality, Na^+, K^+ and Cl^- with respect to day 1 of oral administration. In other investigation reported by Gasparotto Junior et al. (2011), the electrolyte content of Na^+ and K^+ increased although there was no significant value found during day 1, 5, 6 and 7 after administration of *Tropaeolum majus* extract. Other than that, Kazama et al. (2012) also signified that, administration of ethanol extract of *Pereskia grandifolia* produced noteworthy increased of urine and sodium content over 7 days.

On the other part, Lahlou et al. (2007) reported that tansy *(Tanacetum vulgare)* and caraway *(Carum carvi)* extracts significantly increased the urine excretion from day 1 to day 5 and then it became unwavering until day 8. In addition, the authors found that Na^+ excretion at day 2 to 3 was unchanged when compared to day 1. However, later the Na^+ level significantly increased throughout the study period. Contrarily, the K^+ excretion of tansy extract statistically increased throughout 8 days of repeated administration. While caraway extract had no changed on K^+ excretion throughout day 2 to 8, compared to day 1 of administration. Besides that, during the prolonged study for five days there was no such acute sign of toxicity effect was observed, except the presence of slightly soft stool during treatment of CAE at doses of 600, 700 and 800 mg/kg and at 40 and 60 mg/kg during CME treatments at day 4 and 5. Other than that, Mirza et al. (2003) have reported that principal constituent of this plant is not harmless. Besides, it has been proven with the histopathological study on liver and kidney of rats that had been administered with cornsilk extract is safe to be consumed (Mirza et al. 2004).

CONCLUSION

The study illustrates that Malaysian cornsilk have shown marked diuretic activity. Then, the ED_{50} of AEC is observed at 454.10 mg/kg. Beneficially, this extract has shown an interesting potassium sparing effect. It appears that Malaysian cornsilk can be used as an alternative natural diuretic agent as it promotes diuresis and does not affecting osmolality of urine.

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