Antioxidant Activity of The Waste Water of Boiled *Zea mays* (Sweet Corn) on The Cob

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ABSTRACT

Zea mays or corn is one of the important foodstuffs in Indonesia. Harvested area was 33 Provence, which in many cities is commonly sold on the street by hawkers as boiled or burned corn. The objective of the research is to study antioxidant activity of the waste water boiled. Sweet corn on the cob was boiled in 20 minutes. The waste water boiled was dried with freeze dryer equipment. Dried extract was screened of phytochemical constituents, and antioxidant activity was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Screening of phytochemicals showed positive results for the presence of flavonoid, alkaloid, saponin, and quinon. Antioxidant activity EC_{50} was 189.8µg/ml. In conclusion the waste water of boiled sweet corn on the cob has antioxidant activity, and maybe the waste water can be used as antioxidant supplement.

Key word: Antioxidant activity, antioxidant supplement, corn on the cob, DPPH assay.

INTRODUCTION

Zea mays (corn) is an important field crop beside paddy as human food energy. It always was monitored by the Indonesian Ministry of Agriculture. The Indonesian Ministry of Agriculture has some programs in place to assist farmers to have better access to high yielding corn and paddy seed since 2007. Harvested area were 33 Provence: Aceh, North Sumatera, Riau, Jambi, South Sumatera, Lampung, Babel, Kepri, Jakarta, West Java, Central Java, Yogyakarta, East Java, Banten, Bali, West Nusa Tenggara, East Nusa Tenggara, West Kalimantan, Central Kalimantan, South Kalimantan, East Kalimantan, Gorontalo, West Sulawesi, Maluku, North Maluku, West Papua, and Papua (Anggoro, 2012).

Corn was cooked as vegetable or as snack. Boiled and burned corns in many cities are commonly sold on the streets by hawkers. Before boiling, top and part the husks of the corn on the cob will be cut, be pulled and discard. The water used again and again for boiled corn on the cob. The boiled water was waste water, and was thrown away.

Free radicals and other ROS are derived either from the endogenous metabolic processes in the human body or from external sources. The former includes mitochondrial respiration, peroxisomal

metabolism, phagocyte activity, arachidonate pathways, inflammation, ischemia, exercise and reactions involving iron and other transition metals. External sources include exposure to radiation, ozone, cigarette smoke, air pollutant and industrial chemicals. Excess free radicals can accumulate, resulting in oxidative stress, and it condition was associated with a number of diseases including atherosclerosis, Alzheimer's disease, cancer, ocular disease, diabetes, rheumatoid arthritis and motor neuron disease (Hajhashemi, 2010).

Antioxidants are used by aerobic organisms to prevent oxidation that can damage the cells during oxygen metabolism. Oxidation can cause a number of diseases including atheroscleorosis, neurodegenerative disorder, cancer, diabetes, inflammatory and aging. Natural antioxidants extract from fruits, teas, vegetables, cereals and medicinal plants have been investigated extensively due to their effectiveness in eliminating free radical and claimed to be less toxic than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). There are many classes of natural antioxidants and they include vitamins such as tocopherols and vitamin C; and phytochemicals such as flavonoids and phenolic acids which are common to all plant sources (Hasanudin, 2012).

The multifaceted aspects of antioxidants and the basic kinetic models of inhibited autoxidation and analyzes the chemical principles of antioxidant capacity assays. Depending upon the reactions involved, these assays can roughly be classified into two types: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET). One of the HAT is 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity assay (DPPH) (Huang, 2005).

Traditionally corn used to increase the production of breast-milk, kidney stone, fever, heart disease, and diuretic (Hutapea, 2000).

Nowadays, more researches about corn were done. The pharmacological effects as antioxidant activities were tested: in the study of free radical scavenging activity and total antioxidant activity of corn silk extracts in benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether, showed percentage inhibition of free radicals among the extracts, methanolic extract of corn silk

with 100 μg concentration gives higher percentage (95.6%) of free radicals scavenging activity than the other extracts. The free radical scavenging activity increases with increase in concentration. At a concentration of 10 μg/ml, methanolic extract obtained 2.5% of free radical scavenging activity and got increased to 95.6% in 100μg/ml of concentration. About total antioxidant activity, methanolic extract exhibited the strongest antioxidant activity (85.2 mg/ml) among all the other extracts (Bhaigyabati, 2011). In evaluation of polyphenol content and antioxidant activities of cornsilk extracts in some selected organic and aqueous by β-carotene bleaching method, 2,2-diphenyl-2-picrylhydrazyl (DPPH.) radical scavenging, superoxide anion (O_2-) radical scavenging and ferric reducing power activity (FRAP). DPPH test showed methanol extract was the strongest electron or hydrogen donor due to the highest DPPH scavenging activity. The IC_{50} of DPPH scavenging activity of the methanol, ethanol, water and ethyl acetate extracts were 140.89 μg/ml, 143.55 μg/ml, 195.21 μg/ml and 411.69 μg/ml respectively (Nurhanan, 2012). A significant amount of phenol and flavonoids content of ethanol-water extract from corn silk (CS extract) were measured by Folin Ciocalteu and $AlCl_3$ assays. The antioxidant properties were estimated by different methods. The percentage of DPPH radical scavenged by CS extract was 92.6 at a concentration of 1.6 mg/ml. IC_{50} of the extract and the standard compounds butylated hydroxytoluene (BHA) and quercetin was 0.59, 0.053, and 0.025 mg/ml, respectively. Iron chelating activity of the extract was less than the standard compounds. CS extract showed nitric oxide-scavenging effect less than the reference agent (quercetin). The extract showed a high reducing ability. According to ferric thiocyanate (FTC) method, the extract showed more than 88% inhibition of linoleic acid peroxidation. It might be concluded that some of the properties of CS in traditional medicine is due to its antioxidant ability (Ebrahimzadeh et al., 2008, Hasanudin et al., 2012). Methanolic and aqueous extracts of Malaysian corn silk contained flavonoids, saponin, tannins, phlobatannins, phenols, alkaloids and cardiac glycosides, and the total phenolic content in aqueous extract was significantly higher (42.71 ±0.87 μg/g of tannic acid equivalent (TAE)) compared to methanolic extract (40.38 ± 1.10 μg/g of TAE) (Solihah, 2012).

The other pharmacological effects were found, water extract of corn silk treatment markedly reduced hyperglycemia in alloxan-induced diabetic mice. The action of corn silk extract on glycaemic metabolism is not via increasing glycogen and inhibiting gluconeogenesis but through increasing insulin level as well as recovering the injured β-cells (Guo et al., 2009, Hasanudin et al., 2012). Two flavonoid glycosides were isolated from n-butanol fraction of methanol extract of corn silk, and were elucidated as by means of different analytical methods such as UV, IR, NMR and MS (ESI) analyses and by comparison with those reported literature for the compounds, as maysin (**I**) (2″-O-α-L-rhamnosyl-6-C-(6-deoxyxylo- hexos-4-ulosyl)-luteolin) and maysin-3′-methyl ether (**II**) (2″-O-α-L-rhamnosyl- 6-C-(6-deoxy-xylo-hexos-4-ulosyl)-luteolin-3′-methyl ether). The antimicrobial studies of maysin (**I**) and maysin-3′-methyl ether (**II**) were studied against twelve bacteria and one yeast. The sensitivity of the compounds (2.0 mg/mL) towards bacteria was compared with that of standard gentamycin (50 μg/mL). Flavonoid glycosides showed wider range of activity towards gram-positive and gram-negative bacteria. Comparatively, compound **I** exerted highest antibacterial activity towards gram positive bacteria than **II**. In comparison with gentamycin, compound **I** showed significantly ($p<0.05$) higher activity against the tested bacteria except towards *Ent. aerogenes, S. paratyphi* and *P. mirabilis* where it exerted statistically ($p<0.05$) similar activity with gentamycin. Towards *P. aeruginosa*, it showed lower activity than gentamycin (Nessa, 2012). Methanol cornsilk extract has protective effects on nephrotoxicity in rat that was induced by gentamycin (GM) i.p. administration. At the dose 200 and 300 mg/kg of extract gave significantly decreased serum creatinine, but not urea, levels compared with GM group. Co-treatment of corn silk with GM considerably decreased the interstitial nephritis, but not acute tubular necrosis and hyaline casts formation, compared with the GM group. Also, high dose of corn silk caused hyaline cast formation, apoptosis, congestion, and swelling of renal tubules (Sepehri et al., 2011, Hasanudin et al., 2012). The methanolic extract of corn silk at 200, 300 and 400 mg/kg concentrations were tested to the hyperthyroid condition in Swiss albino rats that were induced orally by thyroxine. The evaluation were measured the level of thyroid hormone serum and lipid

profile before and during the experiment and were confirmed with the histopathological study of the thyroid gland. The highest effectiveness concentration is 400 mg/kg which is equal to that of standard drug 0.04% of methimazole (Bhaigyabati, 2012).

METHOD

Material:

Sample : fresh sweet *Zea mays* (corn) on the cob from Lembang (Bandung) (West Java), has length 18–19 cm, diameter 5–6 cm. was harvested in Desember 2012.

Cut off 5 cm the top portion of the cob, and the husks were pulled off of the corn (left the last husk that coat around the corn and did not remove silk).

Reagent : DPPH (Sigma Aldrich), methanol (E.Merck). Demineralization water

Equipment : Freeze dryer Eyela FD-81, Spectrophotometer Jenway 6305 UV-Vis single beam

Experimental Details:

Boiling corn on the cob:

25 corns divided to 5 groups, each group was boiled with 1800 ml boiled water in 20 minutes, and removed the cooked corns from the hot water. The water waste boiled was collected and was dried by freeze dryer: Eyela FD-81.

Phytochemical screening of dried boiled water extract:

The dried extract was analyzed for the presence of phytochemicals such as flavonoid, alkaloid, saponin, quinon, tannin, and steroid/terpenoid using standard procedure (Harbone, 1973).

Preparation of DPPH stock solution:

1.0×10^{-3} M concentration of DPPH solution was prepared in methanol.

Preparation of dried corn extract:

The dried corn extract was dissolved in methanol, and were made in 5 different concentrations: 0.669, 0.446, 0.223, 0.111, 0.056 g/ml.

Assay of antioxidant activity with 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH):

Each of the samples was mixed with 1 ml DPPH stock solution, kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer Jenway 6305 UV-Vis single beam. The absorbance was recorded. The result is % effective inhibition, and was calculated by the equation below:

$$\text{EC \%} = \frac{reference\ absorbance - sample\ absorbance}{reference\ absorbance} \times 100\%$$

RESULT AND DISCUSSION

The weight of dried extract of waste water of boiled corn on the cob = 35.6585 g.

Table 1: Phytochemical Constituents of Waste Water Dried Extract of Boiled Corn on the Cob

No.	Constituents	Results
1.	Alkaloid	+
2.	Flavonoid	–
3.	Saponin	+
4.	Quinon	+
5.	Tannin	–
6.	Steroid/Triterfenoid	–

+ = Present; – = Absent

Table 2: Percent inhibition of Waste Water Dried Extract of Boiled Corn on the Cob on DPPH

No. of samples	Concentration of samples (mg/ml)	Absorbance				% Inhibition
		1	2	3	Average	
Blanc	0	1.003	1.004	1.004	1.004	0
1	0.669	0.295	0.227	0.251	0.258	74.30
2	0.446	0.174	0.178	0.283	0.211	78.98
3	0.223	0.317	0.323	0.321	0.320	68.12
4	0.111	0.591	0.580	0.597	0.589	41.33
5	0.056	0.684	0.748	0.744	0.725	27.78

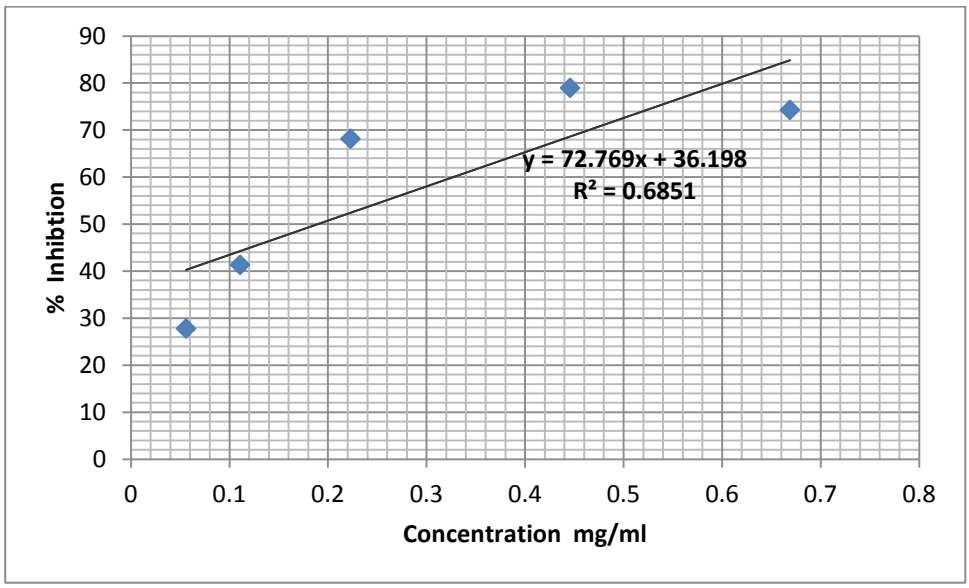

Figure 1: Percent inhibition of Waste Water Dried Extract of Boiled Corn on the Cob on DPPH

The linier regression is y = 72.76x + 36.19

Note : y is % inhibition and x is sample concentration

y = 72.76x + 36.19

50 = 72.76x + 36.19

EC_{50} = 0,1898 mg/ml

The effective concentration of waste water of boiled corn on the cob is 0,1898 mg/ml.

CONCLUSION

The waste water of boiled corn on the cob has antioxidant activity, EC_{50} is 0,1898 mg/ml. Therefore, maybe the waste water can be used as antioxidant supplement.

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