Phytochemical Contents and Antioxidant Activity of Bagasse Sugarcane Extracts

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Abstract:

The objective of this work is to determine phytochemical contents including total phenolic, total flavonoids and total triterpenoid in ethanolic extracts of 2 varieties of sugarcane bagasse. The results showed that the phytochemicals were variable in contents by the sugarcane variety. The highest contents of phytochemical were found in Khon Kaen II extracts which resulted to highest antioxidant activity as well. All phytochemicals were positively corelated to antioxidant activity, especially the free radical scavenging activity when tested by DPPH and ABTS assays and also corelated to reducing power activity by FRAP with modulate value. The obtained results indicated that bagasse is a natural good source of phytochemicals composed of antioxidant activity which would be developed these extracts for further health benefit products.

Keywords: Sugarcane bagasse, Phytochemical Crude extract, Antioxidant activity

Introduction

In recent, study on phytochemicals has been gradually increased. Among the phytochemicals, phenolic compounds are the largest group in plant metabolites [1]. Different types of phenolic compounds including phenols, flavonoid, tannin [2] and terpenoids such as carotenoids [3] have been reported. It has been proved that phytochemicals have various biological activities. Flavonoid is an important substance for antioxidation and could be protected degenerative diseases causing from free radicals [4]. Phytosterols helped to decrease lipid in human blood. Carotenoids and alkaloids have anti-inflammatory which used as active ingredient for various diseases protection [3, 5].

Sugarcane (*Saccharum officinarum* L.) is a main economic crop of many countries includes Thailand. It is planted in all parts of Thailand, especially in the northeastern area. However, the main application of sugarcane is sugar production since the sugarcane composes of high sucrose content (17-35%). Sugarcane was also used for ethanol production as fuel instead of petroleum. Moreover, some reports about phytochemicals in sugarcane have been discovered [6]. The phytochemicals were varied following strain and geographic area planted [7-9].

In the sugar production process, the residuals after juice extraction are bagasse. This bagasse was limited to apply for value added productions and still remaining as waste which gradually increased every year. Therefore, the authors are interesting for studying the phytochemicals in bagasse extract as well as their biological activities. The obtained results would be used as basic information for further studying and value added of this waste.

Materials and methods

Materials

The bagasse of 2 strains of sugarcane for this work are Khon Kaen I and Khon Kaen II which planted in Burirum Province. The bagasse was obtained from Burirum Sugarcane Factory. The sugarcane

tree was placed into manual crusher for juice extraction at room temperature to obtain the bagasse and then dried in an oven at 60 °C for 18 h. The bagasse was grinded into small pieces and kept in a seal bag at room temperature.

Preparation of crude extract

The crude extract of bagasse was extracted by ethanol. The 1g of bagasse was weighed and 25 mL of ethanol was then added into the bagasse. The mixture contained in volumetric flask was shaken for 48 h. All samples were extracted in triplicate. The extracts were pooled and evaporated the solvent by rotary evaporator. The powder of extract was separated from the round bottom bottle and weighed using balance. The exactly dried weight of crude extracts was weighed before adding ethanol for dissolving the prepared crude extract.

Total phenolic content

The total phenolic content (TPC) was determined using a modified colorimetric method [10]. A 1 mL of crude solution was mixed with 5 mL of 10% Folin-Ciocalteu reagent, before incubating at room temperature for 5 min. After that, 4 mL of 7.5% of Na_2CO_3 solution was added into the mixture solution before standing at room temperature for 1 h. Then, the mixture was measured at 765 nm using UV-Vis spectrophotometer. Gallic acid was used as standard for a calibration curve. The TPC was indicated as mg gallic acid equivalent (mgGAE)/100g of dried weight.

Total flavonoid content

The total flavonoid content (TFC) of the bagasse extract was measured using a modified previous method [11]. Briefly, 2 mL of crude solution was mixed with 0.4 mL of distilled water and 0.4 mL of 5% (w/v) $NaNO_2$ was subsequently added. The mixture was then incubated at room temperature for 6 min before adding 0.6 mL of 10% $AlCl_3$, then standing for 6 min. The mixture solution was then mixed with 4 mL of 0.1 M NaOH and left for 15 min at room temperature. The absorbance at 510 nm was measured using UV-Vis spectrophotometer. Quercetin was used as standard for a calibration curve. The TFC was indicated as mg quercetin equivalent (mgQE)/100g of dried weight.

Total triterpenoid content

The total triterpenoid content (TTC) was determined using a modified method [12]. A 0.3 mL of crude solution was evaporated at water bath at 100 °C, then 0.5 mL vanillin-acetic acid (5:95 w/v) and 0.8 mL perchloric acid ($HClO_4$) was added before incubating at 60 °C for 15 min. After that, 5 mL of acetic acid (CH_3COOH) was added into the mixture solution before standing at room temperature for 15 min. The mixture solution was then measured at 548 nm using UV-Vis spectrophotometer. Ursolic acid was used as a standard for a calibration curve. The triterpenoid content was indicated as mg ursolic acid equivalent (mgUA)/100 g dried weight.

Antioxidant activity by DPPH assay

DPPH assay was carried out to measure the free radical scavenging activity following previous method [13]. A 0.5 mL of crude solution was concentrated in methanol followed by mixing with of 0.1 mM DPPH solution in methanol. After incubation at room temperature in the dark for 30 min, the absorbance was read at 517 nm. Trolox (6-hydroxy-2,5,7,8-tetramethychlorman-2-carboxylic acid) was used as positive control for comparison and solvent mixed with 0.1 mM DPPH solution was taken as negative control. The percent scavenging was calculated by following formula

$$\text{DPPH radical scavenging (\%)} = [(A_0 - A_s)/A_0] \times 100$$

where A_0 of control is the absorbance of the solvent mixed with DPPH solution and A_s is the absorbance of the extract solution. DPPH radical scavenging was indicated as mg Trolox equivalent (mgTE)/g dried weight.

Antioxidant activity by ABTS assay

The ABTS assay was performed following the method of Trolox equivalent antioxidant capacity (TEAC) [14]. The stock solution included a 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) solutions were mixed. The working solution was then prepared by adding 10 mL $K_2S_2O_8$ to 10 mL ABTS solution. The two solutions were mixed well and allowed to react for 16 h at room temperature in the dark. The absorbance of solution at 0.7 ± 0.02 was used as working solution. Trolox was used as positive control for comparison. Crude solution (0.5 mL) was allowed to react with 1 mL ABTS working solution in the dark at room temperature for 5 min, and then the absorbance was measured at 734 nm using UV-Vis spectrophotometer. The results were expressed as mg Trolox equivalent (mgTE)/g dried weight.

Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was conducted according to previous method [14]. The working solution was prepared by mixing 25 mL of acetate buffer pH 3.6 (3.1 g of $CH_3COONa.3H_2O$ and 16 mL of CH_3COOH), 2.5 mL TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution and incubating at 37 °C before use. Crude extracts as samples or distilled water as blank (200 µL) were allowed to react with 2.8 mL of the working solution for 30 min in dark at 37°C. Absorbance was measured at 593 nm using UV-Vis spectrophotometer. Ferus sulfate ($FeSO_4$) was used as standard to establish a standard curve. The FRAP antioxidant activity was expressed as mmol of Fe^{2+} equivalents per g of dried weight (mM $FeSO_4$/ g DW).

Statistical analysis

Statistical analyses are performed using Excel software (Microsorft Office 2013) for calculating the means and the standard error of the mean. Results are expressed as the mean ± standard deviation (SD). Determination of f-test (one-way ANOVA) and correlation (Pearson correlation coefficient, *r*) on phytochemical and antioxidant activity is analyzed by using SPSS software for Windows (version 19).

Results and discussion

Phytochemical contents

Table 1 showed phytochemical contents found in bagasse extracts. The results indicated that the crude extract of Khon Kaen II had higher phytochemical contents than Khon Kaen I. The TPC, TFC and TTC were 35.19 mg GAE/100 g DW, 101.47 mg QE/ 100 g DW and 7.73 mg QE/ 100 g DW, respectively. These contents higher than Khon Kaen I about 13, 28 and 44%, respectively even the yield of crude extracts from Khon Kaen I was higher than Knon Kaen II about 26.5%.

In general, the obtained phytochemicals from the bagasse have lower than phytochemicals content found in the sugarcane [7-9] about 33%. However, the phytochemicals content in bagasse have the same content as found in partially purified fraction of sugarcane extract [8]. The types and contents of phytochemicals were varied by some factors including planted regions, climates, strain, parts of plants, harvest times, instrument analysis, solvents, method and procedures analysis [9,16,17].

Table 1 Phytochemical contents of sugarcane bagasse crude extracts.

Extracts	Extraction Yield (%)	TPC (mg GAE/100 g DW)	TFC (mg QE/ 100 g DW)	TTC (mg UA/ 100 g DW)
Khon Kaen I	2.256±0.08	30.75±0.38	73.07±2.05	4.34±0.34
Khon Kaen II	1.783±0.02	35.19±1.04	101.47±3.89	7.73±0.57

Antioxidant activity

The antioxidant activity of bagasse crude extracts was shown in Table 2. The results found that Khon Kaen II had higher antioxidant activities than Khon Kaen I. The radicals scavenging of Khon Kaen II

extract for DPPH and ABTS were 17.32 and 150.85 mg TE/ g DW, respectively which were higher than Khon Kaen I extract about 25%. Moreover, the bagasse extract of Khon Kaen II showed higher reducing power of Fe^{2+} (26.59 mM $FeSO_4$/ g DW) than Khon Kaen I extract about 15%. Comparison with previous reports, the reducing power of bagasse extract has closed to sugarcane tree extract fractionated by silica gel column chromatography [8], but the bagasse extract had higher scavenging free radical activity [7]. This might be according to the bagasse extract composed high content of ortho-dihydroxyl polyphenols such as flavonoids (quercetin, catechin myricetin) which could be interacted well with Fe^{2+} via coordinate linkages [17,18]. Moreover, phenolic compounds which composed high hydroxyl groups are the main antioxidant substances in plants [20,21].

Table 2 Antioxidant activity of sugarcane bagasse crude extracts.

Extracts	DPPH assay (mg TE/ g DW)	ABTS assay (mg TE/ g DW)	FRAP assay (mM $FeSO_4$/ g DW)
Khon Kaen I	13.87±0.99	110.07±2.97	22.69±2.22
Khon Kaen II	17.32±0.92	150.85±5.50	26.59±1.56

Correlation analysis

The correlation between phytochemicals and antioxidant activity of the bagasse extract as shown in Table 3. The results indicated that all phytochemicals; TPC, TFC and TTC positively correlated to antioxidant activity. This indicated that all obtained substances synergistic effect on free radicals. Considering each phytochemical about its antioxidation mechanism, the TPC and TFC preferred scavenging activity than reducing power. However, the TFC showed higher ABTS scavenging than DPPH and reducing Fe^{2+}. The TTC has lower antioxidant activity than others. This indicated that the main mechanism on free radicals of the bagasse extract
was scavenging activity. The obtained results were in agree with previously reported [22-24].

Table 3 Correlation (*r*) of phytochemical contents and antioxidant activity of sugarcane bagasse crude extracts.

Factors	TPC	TTC	TFC	$DPPH^{\cdot}$	$ABTS^{+}$	FRAP
TPC	1	.947**	.947**	.957**	.916*	.622
TTC	-	1	.939**	.934**	.822*	.480
TFC	-	-	1	.950**	.967**	.721
$DPPH^{\cdot}$	-	-	-	1	.876*	.533
$ABTS^{+}$	-	-	-	-	1	.860*
FRAP	-	-	-	-	-	1

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Conclusions

Bagasse composed of different phytochemicals containing antioxidant activity. The types and contents of phytochemicals varied by the strain of sugarcane. The crude extract of Khon Kaen II has higher phytochemicals contents and antioxidant activity than Khon Kaen I. The phytochemicals found in the bagasse extracts showed positively related to their antioxidant activity with statistical significance. The mechanism of phytochemicals was clearly acted of free radical scavenging more than reducing power. This work suggested that the bagasse is a good source of some phytochemicals with high antioxidant potential, especially flavonoids and phenolic acids. Therefore, it might be possible to apply this bagasse extract as human health supplement.

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