PHYTOCHEMICALS AND ANTIOXIDATION OF FRACTIONATED SUGARCANE EXTRACTS: SUPHANBURI 50 VARIETY

PHONGSATHORN MOTHAM, ANSAYA THONPHO, PRASONG SRIHANAM*

Department of Chemistry, Faculty of Science, Mahasarakham University, Kantharawichai District, Maha Sarakham 44150, Thailand

*Corresponding author: psrihanam@gmail.com; prasong.s@msu.ac.th

Abstract:

The objective of this work is to fractionate the Suphanburi 50 sugarcane extract using silica gel column chromatography and screen its phytochemical contents and antioxidation in each fraction. The phytochemicals including total phenolic, flavonoids, saponin, proanthocyanidin and condensed-tannin were varied by the fractions depending on the eluting solvents. Moreover, the antioxidation of the fractionated extracts were also varied following the eluting solvents. All phytochemicals were positively corelated to all antioxidation methods, but in variable values. The obtained results indicated that the sugarcane, Suphanburi 50 variety is a natural good source of phytochemicals and exposed antioxidant activity which would be supported health benefit.

Keywords: Sugarcane; Suphanburi 50; Fractionation; Phytochemical; Antioxidation

Introduction

The medicinal plants including vegetables, fruits, herbs and cereals, have been known as the important sources of secondary metabolites known as phytochemicals (Farhadi *et al.*, 2016). They huge groups such as quinines, phenolics, phytosterols, tannins, flavonoids, alkaloids and saponins (King & Young, 1999; Farhadi *et al.*, 2016). These substances are popularly used for health promotion, personalized treatment and disease prevention worldwide (Sen & Chakraborty, 2017). This was according they have various biological activities such as antioxidant, antibacterial, anti-inflammatory, anti-diabetic and anti-aging (Ignea *et al.*, 2013; Guetat *et al.*, 2017; El-Gawad *et al.*, 2019; Elshamy *et al.*, 2019). They were proved already for their safety without side effects compared with synthetic substances and effective in medicinal and nutritional applications (Saucedo-Pompa *et al.*, 2018). Many studies have demonstrated that they significantly prevent some diseases, and reduce some effects of reactions (Meng *et al.*, 2012).

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 north-eastern area. The sugarcane composed high content of sucrose. Therefore, the main application of sugarcane is sugar production. However, phytochemicals in sugarcane have also been studied and reported (Duarte-Almeida *et al.*, 2007; Feng *et al.*, 2014; Naowaset & Srihanam, 2017). Maha Sarakham Province, the central of the north-eastern Thailand, have different varieties of sugarcane planted in the Agricultural Research and Development Centre. As literature reviews, the information about phytochemicals in sugarcane, especially in Suphanburi 50 variety never been reported. Therefore, the goal of this work is to prepare crude extract of the sugarcane for screening their phytochemicals and antioxidation. The crude extract was then partial purified by fractionation throughout silica gel column and eluted by various solvent systems. The

fractionated extracts were then determined for phytochemicals and antioxidation as comparison with the crude extract.

Materials and Methods

Materials

The sugarcane samples in this work is Suphanburi 50, a popular variety for fresh juice drinking. They were kindly supplied from Agricultural Research and Development Center Mahasarakham, Maha Sarakham, Thailand. The sugarcane samples were cut all leaves, crushed juice and dried in an oven at 60 °C for 18 h. The dried sugarcanes were grinded and kept in a seal bag at room temperature.

Preparation of crude extract

The 1 g of dried sugarcane was weighed and 25 mL of ethanol was then added into the sugarcane samples. 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.

Fractionation of the crude extract

The crude extract was loaded on a 60 cm × 4.5 cm i.d. glass column packed with silica gel (60-200 mesh). The column was then eluted with the different polarity of solvent mixtures at a flow rate of 1.0 mL/min. The fractions were eluted by starting with ethyl acetate/methanol in the following ratios successively: 100:0, 75:25, 50:50, 25:75 and 0:100. After that 10 mL of each fraction is collected continuously. The absorbance of each tube was measured at 280 nm using a UV–Vis spectrophotometer to identify each fraction. Sub-fractions were grouped and pooled before concentration using rotary vacuum evaporator. The obtained residues were dissolved in methanol and stored at - 4℃ until analysis.

Total phenolic content

The total phenolic content)TPC(was determined using a modified colorimetric method of Škerget *et al.* (2005) . 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 1h .Then, the mixture was measured at 765 nm using UV-Vis spectrophotometer .Gallic acid was used as standard and results were expressed as mg GAE/ g DW.

Total flavonoid content

The total flavonoid content (TFC) of the bagasse extract was measured using a modified previous method of Jia *et al.* (1999). 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 and results were expressed as mg QE/g DW.

Total saponin content

The total saponin content (TSC) was determined following the method of Hiai *et al*. (1976). Briefly, A 250 µL of standard solution or methanolic extracts 250 µL of 8% vanillin-ethanol solution were mixed. A 2.5 mL of concentrated H_2SO_4 (72%) was then added with the mixture and stand in an ice water bath. The mixture solution was warmed at 60°C for 15 min, and then cooled in ice-cold water to room temperature. The reaction mixture was measured at 560 nm using a UV-Vis spectrophotometer against a blank. The aescin was used as standard and results were expressed as mg AES/g DW.

Total proanthocyanidin content

The total proanthocyanidin content (TPAC) was analyzed via the procedure of Li *et al*. (2006). Each 200 µL methanolic extract solution and 1.5 mL of 4% vanillin-ethanol solution was mixed together before adding 750 µL concentrated HCl. After left for 15 min, the mixture was measured at 500 nm using a UV-Vis spectrophotometer. The catechin was used as standard and results were reported as mg CE/g DW.

Total condensed-tannins content

Total condensed-tannins content (CDT) of bagasse extracts was investigated following the modified methods of Chupin *et al*. (2013). A 0.5 mL of extract was mixed with 4% vanillin-methanol and 1.5 mL of 3 M HCl. The mixture was then stand in dark at room temperature for 15 min before measuring the absorbance at 500 nm. The catechin was used as standard and results were expressed as milligrams mg CE/g DW.

DPPH assay

DPPH assay was carried out to measure the free radical scavenging activity following previous method of Thaipong *et al*. (2006). 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 equation 1.

$$\text{DPPH radical scavenging } (\%) = [(A_0 - A_s)/A_0] \times 100 \qquad (1)$$

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 TE/g DW.

ABTS assay

The ABTS assay was performed following the method of Trolox equivalent antioxidant capacity (TEAC) by Berg *et al*. (1999). 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 (Berg *et al.*, 1999). 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 mM $FeSO_4$/ g DW.

Cupric reducing antioxidant capacity

The cupric reducing antioxidant capacity (CUPRAC) was described by Apak *et al.* (2004). A 500 µL of 10^{-2} M $CuCl_2$ solution was mixed with 500 µL 7.5 x 10^{-3} M neocuproine solution in ethanol and acetate buffer at pH 7.0. The methanolic extract or standard (x µL) and H_2O [(550 - x) µL] were added to the mixture solution. The absorbance was recorded at 450 nm after incubation for 30 min at room temperature using a UV-Vis spectrophotometer. The results were expressed as mg TE/g DW.

Statistical analysis

Results are expressed as the mean ± standard deviation (SD). Determination of correlation (Pearson correlation coefficient, *r*) on phytochemical and antioxidation was analysed using SPSS software for Windows (version 19).

Results and Discussion

Phytochemical contents

Table 1 showed phytochemical contents found in crude and fractionated extracts. The results indicated that the fractionated extracts found higher contents of TPC, TFC and TSC than crude extract, while the SF2M75 (eluted by ethyl acetate/methanol at 25:75 (v/v)) has higher TPAC than crude extract. In case of CDT, the SF2M75 and SF3M50 (eluted by ethyl acetate/methanol at 50:50 (v/v)) have higher CDT than the crude extract. Beside the fractionated extracts, the SF2M75 showed the highest phytochemical contents than other. Among the phytochemicals, TSC is predominant substance, following TFC and TPC, respectively. The results indicated that almost tested phytochemicals found highly after fractionation. This mean that silica gel column could be used to separate some impurity in the crude extract. The CDT found the lowest content in crude extract, but it found in higher content than TPAC in the fractionated fractions. Moreover, the SF4M75 and SF5M100 not detected of the TPAC. In general, the obtained phytochemical contents varied by the eluting fractions. This may be involved the chemical structure of each substances as well as the variable polarity of mobile phase that used for the

fractionation of the crude extract (Zahradníková *et al.*, 2008; Naowaset & Srihanam, 2017). Furthermore, the types and contents of phytochemicals were differed by various factors including instrument, method and procedures analysis (Berli *et al.*, 2012; Feng *et al.*, 2014; Antoniolli *et al.*, 2015).

Table 1: Phytochemical contents in crude and fractionated extracts.

Samples	TPC (mg GAE[a])	TFC (mg QE[a])	TSC (mg AES[a])	TPAC (mg CE[a])	CDT (mg CE[a])
Crude	6.640 ± 0.000	4.524 ± 0.081	30.228 ± 0.051	2.030 ± 0.275	1.537 ± 0.696
SF1M0	-	-	-	-	-
SF2M25	0.520 ± 37.563	69.681 ± 0.818	195.238 ± 2.182	4.022 ± 0.077	13.018 ± 0.215
SF3M50	17.646 ± 1.138	11.575 ± 0.161	49.048 ± 3.299	0.178 ± 0.038	1.778 ± 0.077
SF4M75	10.682 ± 1.217	11.097 ± 0.061	68.907 ± 2.968	ND	0.798 ± 0.133
SF5M100	14.213 ± 0.578	10.230 ± 0.278	176.927 ± 1.427	ND	0.400 ± 0.133

[a]/g DW
ND = not detected

Antioxidation test

The antioxidation of crude and fractionated extracts was shown in Table 2. The results found that the fractionated extracts by methanol 25% (SF2M25) and 50% (v/v) (SF3M50) had higher antioxidation than the crude extract in every tested method. Besides the fractionated extracts, the SF2M25 has the most power antioxidation, and then SF3M50. The SF4M75 and SF5M100 showed lower antioxidation power than the crude extract by DPPH assay, but in higher power by ABTS, FRAP and CUPRAC assays. The radical scavenging activities of the SF2M25 were higher than the crude extract for DPPH and ABTS about 18 folds while metal reducing power activities for FRAP and CUPRAC were about 55 and 30 folds, respectively. The results indicated that the extracts preferred to act as reducing power than radical scavenging. This might be according to the sugarcane extract composed high content of ortho-dihydroxyl polyphenols such as flavonoids and saponin which could be interacted well with Fe^{2+} via coordinate linkages (Andjelkovic *et al.*, 2006; Moran *et al.*, 1997). Moreover, phenolic compounds which composed high hydroxyl groups are good antioxidant (Xia *et al.*, 2011; Visioli *et al.*, 2011; Guendez *et al.*, 2005; Kim *et al.*, 2006; Katalinić *et al.*, 2010).

Correlation analysis

The correlation extinction between phytochemicals and antioxidant activity of the extracts was shown in Table 3. The TPC has high positively correlated all substances, except TSC. Moreover, it showed high positively correlated to every antioxidation assays. TFC showed similar trend of correlation like the TPC and TPAC, but has slightly higher reducing power than the TPC and the same value to TPAC. Surprise, TSC indicated moderated value of positive correlation to all antioxidation assays while CDT showed high positively correlated to all antioxidation assays, except ABTS. This indicated that all tested substances have synergistic effect on oxidants. From the results, most of phytochemicals showed preferably on reducing power than scavenging activity.

Table 2 : Antioxidation of crude and fractionated extracts.

Samples	DPPH (IC50 mg/mL)	ABTS (IC50 mg/mL)	FRAP (µM Fe2+/g DW)	CUPRAC (mg TE/g DW)
Crude	19.819 ± 0.094	3.501 ± 0.330	3.511 ± 0.439	1.185 ± 0.016
SF1M0	ND	ND	ND	ND
SF2M25	1.327 ± 0.028	0.212 ± 0.004	167.052 ± 1.228	31.963 ± 2.135
SF3M50	11.215 ± 0.054	1.857 ± 0.022	21.071 ± 0.614	4.972 ± 0.025
SF4M75	14.914 ± 0.083	8.770 ± 0.176	5.034 ± 0.294	1.393 ± 0.092
SF5M100	16.366 ± 0.119	4.515 ± 0.042	11.000 ± 0.291	3.041 ± 0.035

ND = not detected

Table 3: Correlation extinction (*r*) of phytochemical contents and antioxidation of sugarcane extracts.

List	TPC	TFC	TSC	TPAC	CDT	DPPH	ABTS	FRAP	CUPRAC
TPC	1	0.971**	0.608*	0.976**	0.979**	-0.970**	-0.805**	0.986**	0.987**
TFC		1	0.638*	0.999**	0.996**	-0.953**	-0.650*	0.997**	0.994**
TSC			1	0.633*	0.591*	-0.422	-0.416	0.631*	0.633*
TPAC				1	0.998**	-0.959**	-0.672*	0.999**	0.995**
CDT					1	-0.973**	-0.687*	0.998**	0.995**
DPPH						1	0.758**	-0.966**	-0.964**
ABTS							1	-0.708*	-0.718**
FRAP								1	.997**
CUPRAC									1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Conclusion

Fractionation by silica gel column chromatography could be used for partial purification of the crude extract. The fraction eluted by the mixture of ethyl acetate/methanol at 75:25 ratio obtained the highest phytochemical contents. The highest substance found in the Suphanburi 50 sugarcane extract was TSC, and then TFC and TPC respectively. The phytochemical

contents varied by the eluting solvent. All fractionated extracts showed almost higher antioxidation than the crude extract, except SF1M0. The phytochemical contents were positively correlated to all antioxidation assays with higher reducing power than free radical scavenging. This work suggested that the Suphanburi 50 variety of sugarcane is a good source of phytochemicals, expressed high antioxidant potential. This indicated that the extract of this sugarcane might be used as human health supplement.

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