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Research Article
Antioxidative Compounds Investigation in Sugarcane Bagasse Extracts Fractionated by Silica Gel Column Chromatography

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Abstract

Background and Objective: In the sugar production process, the sugarcane tree which squeezed sucrose already were called bagasse. They have been reported as agricultural sources for phytochemicals and would be applied in health supplement products. The objectives of this work were to fractionate the crude extracts of different sugarcane bagasse cultivars, Authong 17 (AU17) and Suphanburi 72 (SP72) using silica gel column chromatography and investigate their phytochemical contents and antioxidant activities in each fraction. **Materials and Methods:** The two cultivars of sugarcane bagasse were extracted by ethanol before fractionation by silica gel column chromatography. The fractions were collected and then investigated for phytochemicals and antioxidant activities. The individual types and contents of phytochemicals were analyzed by High-Performance Liquid Chromatography (HPLC). **Results:** The total phytochemicals including total phenolic, flavonoids, saponin, proanthocyanidins and condensed-tannin as well as individual phytochemical contents were varied by the fractions depending on the eluting solvents. Moreover, the antioxidant activity of the fractionated extracts was also varied following the eluting solvents. **Conclusion:** The bagasse is a naturally good source of phytochemicals with an antioxidant activity that would be developed from sugarcane bagasse extracts for further health benefit products.

Key words: Phytochemicals, antioxidant activity, cultivar, bagasse, sugarcane

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Free radicals can cause the onset of oxidative stress, which can result in damage to biomolecules and chronic diseases[1]. There are several sources of both intra- and extra-cellular free radicals[2,3]. The study of substances with protective effects against reactive oxygen and free radicals has attracted increasing attention, especially natural products. It is well known that plants, including vegetables, fruits, herbs and cereals, are the main sources of natural antioxidants. Generally, plants produce various secondary metabolites including phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols[4]. Among the phytochemicals, phenolic compounds are the largest group in plant metabolites[5]. Various reports have been proved that phytochemicals have various biological activities and could be protected degenerative diseases causing by free radicals[6-12]. In addition, plant-derived substances have been proven their safety without side effects compared with synthetic substances. Therefore, study on plants phytochemicals are very interested and increasingly performed to recover the new bioactive compounds and their potential information[13-16].

Sugarcane (*Saccharum officinarum* L.) is an important economic crop in many countries including Thailand. It is planted in all parts of Thailand, especially in the northeastern area. However, the main application of sugarcane is sugar production since sugarcane has a high sucrose content. Sugarcane has also been used for ethanol production as fuel instead of petroleum. Moreover, sugarcane is composed of many types of phytochemicals[17]. The phytochemicals found were varied depending upon strain and geographic area in which the crop was planted[18]. In the sugar production process, the residual after juice extraction is bagasse. This bagasse has limited application for value-added productions and remains as waste which gradually increases every year. In previous works, we found that the sugarcane bagasse composed of high phytochemical contents as same as the sugarcane tree[19]. Therefore, the authors are interested in fractioning the crude extracts of bagasse using silica gel chromatography. The fractions were then investigated for the phytochemicals and antioxidant activity. Finally, the contents of each phytochemical were analyzed by HPLC.

MATERIALS AND METHODS

Study area: This work done for six months from November, 1, 2020, to April 30, 2021. The experiment was performed at the Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Materials: The 2 cultivars of sugarcane bagasse, Authong 17 (AU17) and Suphanburi 72 (SP72) were purchased from a farmer in MahaSarakham province, Thailand. The sugarcane bagasse samples were cut into small pieces, dried in an oven at 60°C for 18 hrs. The dried sugarcanes were ground and kept in a sealed bag at room temperature.

Methods

Crude extraction: The 15 g of sugarcane powder was immersed in 300 mL of ethanol mixed hydrochloric acid (99:1 v/v) contained in a volumetric flask and then extracted by sonication for 3 hrs. All samples were extracted in triplicate. The extracts were pooled and evaporated with the solvent by a rotary evaporator. The dried crude extracts were dissolved by ethanol and stored in a freezer until analysis.

Fractionation of the crude extract: The crude extract was loaded on a 60×4.5 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^{-1}. 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 a rotary vacuum evaporator. The obtained residues were dissolved in methanol and stored at -4°C until analysis.

Total phenolic content: The Total Phenolic Content (TPC) of the extracts was determined following the previous method[20]. The crude and fractionated extracts were mixed with Folin-Ciocalteu reagent mixed 7.5% Na_2CO_3 solution. After standing for 30 min, the mixture was measured absorption at 765 nm and gallic acid was used as standard.

Total flavonoid content: The Total Flavonoid Content (TFC) was determined following the previous method[21]. The crude and fractionated extracts were mixed with distilled water, 5% $NaNO_2$ solution and 10% $AlCl_3$ solution. Finally, a 1 M NaOH solution was added into the mixture and left to stand for 15 min. The absorbance at 510 nm was measured and catechin was used as standard.

Total saponin content: The Total Saponin Content (TSC) was determined following the previous method[19]. The crude and fractionated extracts were mixed with 8% vanillin-ethanol and

concentrated H_2SO_4 (72%) before warming at 60°C for 15 min. After that, the mixture solution was cooled in ice-cold water to room temperature and then measured at 560 nm and aescin was used as standard.

Total condensed-tannins content: Total condensed-tannins content (CDT) was investigated following the previous method[22]. The crude and fractionated extracts were mixed with 4% vanillin-methanol and 3 M HCl and then stand in dark at room temperature for 15 min before measuring the absorbance at 500 nm. The catechin was used as standard.

DPPH radical scavenging activity: The DPPH• scavenging activity of the crude and fractionated extracts was determined according to a previous method[23]. The absorbance was detected at 517 nm and percent inhibition of the DPPH activity was calculated following Eq. 1:

$$\text{DPPH inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where, A_C is absorbance of the control (blank) and A_S is absorbance of the extract. The antioxidant activity represented by the 50% inhibition (IC_{50}) value.

ABTS radical scavenging activity: The ABTS radical scavenging activity was determined following the previous method[20]. A 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was mixed with $K_2S_2O_8$ solution to generate ABTS•+and the absorbance at 734 nm was adjusted by distilled water to 0.700±0.020. The crude and fractionated extracts were mixed with ABTS•+solution in the dark for 6 min before measuring at 734 nm. The percent inhibition of ABTS•+scavenging activity was calculated by following equation 1 and the antioxidant activity represented by the 50% inhibition (IC_{50}) value.

Ferric reducing antioxidant power: The reducing activity of the crude and fractionated extracts was determined by the FRAP method[24]. The FRAP reagent (mixture of acetate buffer (pH 3.6), 20 mM $FeCl_3$ and 150 µL 10 mM TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine) in 40 mMHCl) was mixed with the extracts and then incubated for 15 min at 37°C. The absorbance at 593 nm was measured and expressed results as µmol Fe^{2+}/g DW.

Identification and quantification of phenolic compounds: The phenolic constituents of methanolic extracts were distinguished by HPLC-UV system with a reversed-phase column Inertsil ODS-3, C18 (4.6×250 mm, i.d., 5 µm particle size) with Shimadzu LC-20AC pumps (Shimadzu Co., Kyoto, Japan), SPD-M20A and a diode array detector. The conditions used following the previous report[21]. Elution was carried out by mobile phase comprised of deionized water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B), at a flow rate of 0.8 mL min^{-1}. The elution was performed by gradient system between solvent A and solvent B as follows: from 0-5 min (5-9% solvent B), from 5-15 min (9% solvent B), from 15-22 min (9-11% solvent B), from 22-38 min (11-18% solvent B), from 38-43 min (18-23% solvent B), from 43-44 min (23-90% solvent B), from 44-45 min (90-80% solvent B), from 45-55 min (isocratic at 80% solvent B), from 55-60 min (80-5% solvent B) and a re-equilibration period of 5 min with 5% solvent B used between individual runs. The column temperature was maintained at 38°C and 20 µL injection volume was adjusted. The UV-diode array detection was set at 280 nm (hydroxybenzoic acid, gallic acid, catechin, epicatechin), 320 nm (hydroxycinnamic acid, caffeic acid, p-coumaric acid, ferulic acid), 306 nm (stilbene, resveratrol) and 360 nm (flavonols, quercetin, rutin, myricetin). Phenolic compounds in the samples were identified by comparing their relative retention times and peak areas and UV spectra with those of authentic compounds and were detected using an external standard method.

Statistical analysis: The Mean±standard deviation (SD) and Duncan's new multiple range tests were used to evaluate the significant differences with $p<0.05$ and $p<0.01$.

RESULTS

Phytochemical contents: Table 1 shows phytochemical contents found in crude and fractionated extracts of sugarcane bagasse. The results indicated that all tested substances were varied by cultivars and sub-fractions. For AU17, the TPC in crude (12.13±0.33 mg g^{-1} GAE) generally found higher than the fractionated extracts (2.44-4.09 mg g^{-1} GAE). Among the finding fractions, the SF4M75 fraction showed the highest TPC (4.09±0.72 mg g^{-1} GAE). The TFC found in the fractionated extracts (13.19-27.58 mg g^{-1} QE) higher than the crude extract (10.88±0.03 mg g^{-1} QE). The SF5M100 fraction has the highest TFC (27.58±0.34 mg g^{-1} QE). The TSC was found in the fractionated extracts (186.19-199.52 mg g^{-1} AES) higher than the crude extract (43.04±0.13 mg g^{-1} AES), except SF5M100 fraction (38.57±0.86 mg g^{-1} AES), which was lower than the crude extract. The crude extract has TPAC (3.35±0.19 mg g^{-1} CE) higher than the fractionated extracts

Table 1: Phytochemical contents in the sugarcane bagasse crude and fractionated extracts

Extracts	TPC (mg g^{-1} GAE)	TFC (mg g^{-1} QE)	TSC (mg g^{-1} AES)	TPAC (mg g^{-1} CE)	CDT (mg g^{-1} CE)
AU17					
Crude	12.13±0.33^{c}	10.88±0.03^{b}	43.08±0.13^{c}	3.35±0.19^{c}	2.75±0.76^{c}
SF1M0	-	-	-	-	-
SF2M25	-	-	-	-	-
SF3M50	3.86±0.27^{b}	26.66±0.48^{d}	199.52±0.18^{e}	1.84±0.04^{b}	1.56±0.05^{b}
SF4M75	4.09±0.72^{b}	13.19±0.36^{c}	186.19±0.18^{d}	0.49±0.04^{a}	3.29±0.08^{d}
SF5M100	2.44±0.15^{a}	27.5±0.34^{d}	38.57±0.86^{b}	0.33±0.07^{a}	3.42±0.08^{d}
SP72					
Crude	8.11±0.28^{d}	10.03±0.03^{b}	29.99±0.05^{a}	2.20±0.08^{c}	2.68±0.03^{c}
SF1M0	-	-	-	-	-
SF2M25	-	-	-	-	-
SF3M50	14.23±0.29^{e}	5.23±0.16^{a}	194.29±0.55^{e}	0.47±0.13^{a}	1.42±0.05^{b}
SF4M75	24.62±0.33^{f}	5.47±0.12^{a}	437.68±0.76^{f}	ND	0.71±0.08^{a}
SF5M100	34.05±0.74^{g}	10.45±0.12^{b}	36.19±0.30^{b}	0.18±0.04^{a}	0.81±0.05^{a}

Results are expressed as mean±SD of triplicate measurements. Means with different letters in the same column represent significant differences at $p<0.05$. (- means not detected). AU17: Authong 17, SP72: Suphanburi 72, TPC: Total phenolic content, TFC: Total flavonoid content, TSC: Total saponin content, CDT: Total condensed tannin content, TPAC: Total proanthocyanidins content, SF1M0: Sub-fraction one without methanol, SF2M25: Sub-fraction two with 25% methanol (v/v), SF3M50: Sub-fraction three with 50% methanol (v/v), SF4M75: Sub-fraction four with 75% methanol (v/v), SF5M100: Sub-fraction five methanol, GAE (mg g^{-1}): Milligram per gram gallic acid, QE (mg g^{-1}): Milligram per gram quercetin, AES (mg g^{-1}): Milligram per gram aescin, CE (mg g^{-1}): Milligram per gram catechin

(0.33-1.84 mg g^{-1} CE). The CDT found in the fractionated fractions SF5M100 (3.42±0.08 mg g^{-1} CE) and SF4M75 (3.29±0.08 mg g^{-1} CE) in higher content than the crude extract (2.75±0.76 mg g^{-1} CE). For SP72, the oxidative substances in the crude extract, TPC (8.11±0.28 mg g^{-1} GAE), TFC (10.03±0.03 mg g^{-1} QE), TSC (29.99±0.05 mg g^{-1} AES), TPAC (2.20±0.08 mg g^{-1} CE) and CDT (2.68±0.03 mg g^{-1} CE) were found in lower content than in the crude extract of AU17. The TPC (14.23-34.05 mg g^{-1} GAE) and TSC (39.19-437.68 mg g^{-1} QE) in the fractionated extracts were higher than the crude extract. The highest TPC was found in SF5M100 (34.05±0.74 mg g^{-1} GAE), while TSC was found in SF4M75 (437.68±0.76 mg g^{-1} AES), respectively. TFC found the highest in SF5M100 (10.45±0.12 mg g^{-1} QE) with similar content as found in the crude. The CDT found in the fractionated extracts arranged from 0.71-1.42 mg g^{-1} CE were lower than that of crude extract.

Antioxidant activity: Table 2 showed antioxidant activity of the extracts determination by different methods. In AU17, both SF3M50 and SF4M75 fractions have lower IC_{50} values of 4.86±0.02 and 8.53±0.22 μg mL^{-1}, respectively. These values were lower than that of the crude extract (11.13±0.04 μg mL^{-1}) and control (8.57±0.05 μg mL^{-1}). This means the fractionated extracts have higher potential antioxidant activity than crude and Trolox. ABTS assay showed that the fractionated extracts have IC_{50} values in the range of 48.47-697.95 μg mL^{-1}. Among the fractionated extracts, SF3M50 has the lowest IC_{50} value (48.47±1.32 μg mL^{-1}) which was lower than the crude extract (249±0.08 μg mL^{-1}). All fractionated extracts have FRAP values in the range of 26.89-85.54 μM Fe^{2}+g^{-1} DW. These obtained values were higher than that of crude extract (20.41±1.54 μM Fe^{2}+g^{-1} DW). The CUPRAC assay showed results of the SF3M50 and SF5M100 fractions were 8.39±0.33 and 5.69±0.23 mg TE g^{-1} DW, respectively. The obtained CUPRAC values were higher than the crude extract (5.60±0.11mg TE g^{-1} DW). In SP72, the scavenging DPPH and ABTS free radicals have higher but FRAP and CUPRAC values were lower than the AU17. With DPPH, SF3M50 fraction has an IC_{50} value of (7.88±0.10 μg mL^{-1}) which was lower than the crude (14.11±0.27 μg mL^{-1}) and Trolox(8.57±0.05 μg mL^{-1}). All fractionated extracts showed IC_{50} values by ABTS assay in the range of 1910.76-1935.73 μg mL^{-1} which were lower than the crude extract (2388±0.03 μg mL^{-1}). However, the obtained values were higher than Torox (3.21±0.05 μg mL^{-1}). FRAP values of fractionated extracts were in the range of 1.76-5.91 μM Fe^{2}+g^{-1} DW which were lower than in the crude extract (13.31±0.67 μM Fe^{2}+g^{-1} DW). The crude extract showed a CUPRAC value of 3.29±0.18 mg TE g^{-1} DW which was higher than that of the fractionated extracts (1.36-2.26 mg TE g^{-1} DW).

Quantity analysis of phenolic compounds: The types and contents of the phenolic compounds shown in Table 3. The AU17 crude extract composed high content of gallic acid, p-coumaric acid, caffeic acid, resveratrol and ferulic acid for phenolic acids. Among them, gallic acid (1.50 mg g^{-1} DW) was the highest content in the crude extract. The major flavonoid substances in the crude of AU17 were quercetin

Table 2: Antioxidant activity in the sugarcane bagasse crude and fractionated extract

Extracts	DPPH (IC_{50} μg mL^{-1})	ABTS (IC_{50} μg mL^{-1})	FRAP (μM Fe^{2+} g^{-1} DW)	CUPRAC (mg TE g^{-1} DW)
AU17				
Crude	11.13±0.04^{c}	249.00±0.08^{a}	20.41±1.54^{e}	5.60±0.11^{e}
SF1M0	-	-	-	-
SF2M25	-	-	-	-
SF3M50	4.86±0.02^{a}	48.47±1.32^{a}	50.42±0.80^{e}	8.39±0.33^{e}
SF4M75	8.53±0.22^{b}	366.92±1.81^{b}	85.54±1.79^{g}	4.75±0.10^{d}
SF5M100	12.86±0.11^{d}	697.95±3.82^{c}	26.89±1.18^{c}	5.69±0.23^{e}
SP72				
Crude	14.11±0.27^{e}	2388.00±0.03^{a}	13.31±0.67^{d}	3.29±0.18^{c}
SF1M0	-	-	-	-
SF2M25	-	-	-	-
SF3M50	7.88±0.10^{b}	1910.79±1.73^{d}	2.29±0.15^{a}	2.15±0.48^{b}
SF4M75	18.04±0.07^{e}	1933.22±2.74^{d}	1.76±0.04^{a}	2.26±0.17^{b}
S^{e}5M100	24.49±0.01^{g}	1935.73±2.37^{d}	5.91±0.13^{b}	1.36±0.01^{a}
Trolox	8.57±0.05^{b}	3.21±0.05^{a}	-	-

Results are expressed as mean±SD of triplicate measurements. Means with different letters in the same column represent significant differences at p<0.01. (- means not detected). μg mL^{-1}: Microgram per milliliter, μM Fe^{2+} g^{-1} DW: Micromolar ferrous ion per gram dry weight, mg TE g^{-1} DW: Milligram trolox per gram dry weight

Table 3: Types and contents (mg g^{-1} DW) of phytochemical substances analyzed by HPLC

Extracts	Gallic acid	Catechin	Caffeic acid	Epicatechin	p-coumaric acid	Ferulic acid
AU17						
Crude	1.50±0.00^{c}	-	0.56±0.00^{b}	0.23±0.02^{b}	0.96±0.04^{b}	0.32±0.00^{a}
SF3M50	0.978±0.00^{b}	0.04±0.00^{a}	0.59±0.00^{b}	0.52±0.02^{b}	1.18±0.63^{b}	0.37±0.02^{a}
SF4M75	1.05±0.00^{b}	-	-	-	0.13±0.00^{a}	0.34±0.00^{a}
SP72						
Crude	0.47±0.82^{a}	0.03±0.00^{a}	0.55±0.00^{b}	0.16±0.02^{b}	0.54±0.03^{a}	0.30±0.00^{a}
SF4M75	1.62±0.00^{c}	-	0.19±0.03^{a}	0.05±0.00^{a}	0.31±0.00^{a}	0.31±0.00^{a}
SF5M100	0.48±0.00^{a}	-	-	-	0.13±0.00^{a}	-

Extracts	Rutin	Myricetin	Resveratrol	Quercetin
AU17				
Crude	0.01±0.00^{a}	0.01±0.00^{a}	0.43±0.02^{a}	0.43±0.02^{a}
SF3M50	0.05±0.00^{a}	0.02±0.01^{a}	8.39±1.40^{b}	4.25±1.62^{b}
SF4M75	-	-	0.31±0.00^{a}	0.80±0.00^{a}
SP72				
Crude	-	0.02±0.00^{a}	0.25±0.01^{a}	0.45±0.01^{a}
SF4M75	0.01±0.00^{a}	0.01±0.00^{a}	0.20±0.00^{a}	0.17±0.03^{a}
SF5M100	-	-	0.32±0.00^{a}	0.21±0.03^{a}

Results are expressed as mean±SD of triplicate measurements. Means with different letters in the same column represent significant differences at p<0.01. (- means not detected): mg g^{-1} DW: Milligram per gram dry weight

(0.43±0.02 mg g^{-1} DW) and epicatechin (0.23±0.02 mg g^{-1} DW). In the fractionated extracts, SF3M50 composed higher types and content of phenolic compounds than the SF4M75. The main substances were resveratrol, caffeic acid, p-coumaric acid, content of phenolic compounds than the SF4M75. The main substances were resveratrol, caffeic acid, p-coumaric acid, ferulic acid and gallic acid. Among the phenolic acids, resveratrol (8.39±1.40 mg g^{-1} DW) was the substance obtaining the highest content, while quercetin (4.25±1.62 mg g^{-1} DW) was the main flavonoid in this fraction. Considering phenolic acid in SP72 extracts, caffeic acid (0.55 mg g^{-1} DW) was the main substance in the crude extract, while quercetin (0.45±0.01 mg g^{-1} DW) was the main flavonoid. The SF4M75 fraction found the oxidative substances in higher types and contents than SF5M100. Gallic acid (1.62 mg g^{-1} DW) was the highest substance and found higher than in the crude extract. Quercetin (0.17±0.03 mg g^{-1} DW) was the main flavonoids in this fraction and found in lower content than the crude extract.

DISCUSSION

Previous reports have been suggested that the phytochemical types and contents were varied by cultivars and parts, geography, climate, harvesting time, season, methods and instrument for analysis[18,25-39]. In addition, the extraction process and solvent use were also the main influencer for obtaining the types and contents of the phytochemicals[30]. In general, almost tested phytochemicals found in the fractionated extracts by silica gel column than in

the crude extract. This indicated that the fractionation method helped to concentrate on the phytochemical content.

In this work, many antioxidant activity methods were selected since it is not enough method clarifying the antioxidant activity of the extract[31]. Both free radical scavenging and metal-reducing power were performed[19,32]. The antioxidant activity results indicated that the fractionated extract of AU17 showed higher antioxidant activity potential than SP72. However, the activity mechanism was variable profiles. The variable might be involved with the types and contents of phenolic compounds as well as the functional groups in their structures. It is well known that the position of leaving groups like hydroxyl (-OH) ortho-dihydroxyl and adjacent double bond in carbon ring affected the antioxidant activity of the compounds[29,33]. Flavonoids and saponin composed of ortho-dihydroxyl polyphenols were well known for their chelating properties via coordinate bond[34]. Phenolic acids that composed of hydroxyl groups in their structure were also known as good electron leaving groups[31,34-37]. High-Performance Liquid Chromatography (HPLC), a popular method for identification and quantification of natural products[38,39] was selected for analysis of the bagasse extracts. Those of phenolic acids and flavonoids were analyzed due to their biological activities[12,40], especially pharmaceutical activity[41]. The main substances, gallic acid, p-coumaric acid, caffeic acid, resveratrol and ferulic acid, quercetin and epicatechin were observed. These obtained substances were in agreement with the previous reported[12,42]. However, this work found resveratrol in high content which was conversely obtained by previous reported[20,43]. Therefore, this finding confirmed that the types, contents and profiles of phytochemicals were varied by various factors as suggested above[18,44].

In the future study, different solvents, methods and other biological activities such as antibacterial, enzyme inhibition effect and antidiabetic would be performed to confirm the potential of the finding compounds in the sugarcane bagasse.

CONCLUSION

The fractionation of the bagasse extracts of two cultivars of sugarcane by silica gel column chromatography showed higher phytochemical contents and antioxidant activity than the crude extract. The phytochemicals as well as the antioxidant varied by the cultivars and the eluted solvents. With HPLC analysis, the large groups of substances found in the fractionated extracts were phenolic acids such as gallic acid, p-coumaric acid, resveratrol caffeic acids and ferulic acid and flavonoids such as quercetin and catechin. The fractionated extracts found high phytochemical contents than the crude extract in all most types of the tested substances.

SIGNIFICANCE STATEMENT

This study of phytochemical contents and antioxidant activity of bagasse extracts fractionated by silica gel column: Effect of different sugarcane cultivars that can be beneficial for further application as health supplement substances or folk medicine. This study will help the researcher to uncover the critical areas of natural products from local wisdom that many researchers were not able to explore. Thus, new information about phytochemicals of sugarcane bagasse may be arrived at.

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