

# Phytochemicals and Antioxidant Activity in Sugarcane (*Saccharum officinarum* L.) Bagasse Extracts

Panadda Sanarat, Phongsathorn Motham, Ansaya Thonpho,  
Prasong Srihanam\*

Department of Chemistry, Faculty of Science, Mahasarakham University,  
Khamriang, Karntarawichai, Maha Sarakham 44150, Thailand

\*Corresponding author e-mail: [prasong.s@msu.ac.th](mailto:prasong.s@msu.ac.th)

Received: 3 Dec 2020 / Revised: 10 Feb 2021 / Accepted: 24 May 2021

## Abstract

The aim of this work was to screen the phenolic compounds and antioxidant activity of sugarcane (*Saccharum officinarum* L.) bagasse extracts. The selected sugarcane bagasse was cultivated in Maha Sarakham province. Suphanburi 50 (SP50) and 72 (SP72) and Authong 17 (AU17) cultivars were extracted with methanol and solvent evaporated using a rotary evaporator. The methanolic crude extracts were then analyzed for total phenolic (TPC), flavonoid (TFC), saponin (TSC), condensed tannin (TCT), and proanthocyanidin (TPAC) content. It was found that AU17 extract had the highest content of phytochemicals. The AU17 extract also has the highest antioxidant activities, when studied by free radical (ABTS, DPPH) scavenging activity and metal- (FRAP, CUPRAC) reducing power. TPC was positively correlated to DPPH, FRAP, and CUPRAC than that of ABTS, while TFC showed a high correlation using all the tested methods for antioxidant activity. Using HPLC, AU17 bagasse extract showed higher phytochemical contents than SP strains. The dominant substances in the sugarcane extracts were gallic acid, *p*-coumaric acid, caffeic acid, quercetin, and epicatechin. The results suggested that sugarcane bagasse is a potential source of natural phytochemicals and might be of use as a source of substances for health benefits.

**Keywords:** Phytochemical, Antioxidant, Sugarcane, Bagasse, Crude extract

## 1. Introduction

Recently, the study of substances with protective effects against reactive oxygen and free radicals has attracted increasing attention. There are several sources of both intra- and extra-cellular free radicals (Carocho & Ferreira, 2013; Lobo, Patil, Phatak, & Chandra, 2010). Free radicals can cause the onset of oxidative stress, which can result in damage to biomolecules and chronic diseases (Dalle-Donne, Rossi, Colombo, Giustarini, & Milzani, 2006). Therefore, the interest in finding antioxidants has increased greatly, 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 (Alghazeer, El-Saltani, Saleh, Al-Najjar, & Hebail, 2012). They have revealed potent efficiency against free radicals. Moreover, they also have various bioactivities, such as anti-inflammatory, antibacterial,

anticancer, reduce the risk of cardiovascular disease and diabetes (Butsat, Weerapreeyakul & Siriamornpun, 2009). Phenolic compounds in plants have been the subject of many studies for their antioxidant properties (Denev, Kratchanov, Ciz, Lojek, & Kratchanova, 2012). Many studies have demonstrated that they significantly prevent some diseases, and reduce some effects of oxidative reactions (Meng, Fang, Qin, Zhuang, & Zhang, 2012).

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 (17-35%). Sugarcane has also been used for ethanol production as fuel instead of petroleum. Moreover, sugarcane is composed of many types of phytochemicals (Duarte-Almeida, Negri, Salatino, de Carvalho, & Lajolo, 2007). The phytochemicals found were varied depending upon strain and geographic area in which

the crop was planted (Feng, Luo, Zhang, Zhong, & Lu, 2014; Kraphankhieo & Srihanam, 2016; Naowaset & Srihanam, 2017).

In the sugar production process, the residual after juice extraction is bagasse. This bagasse has limited application for value added productions and still remains as waste which gradually increases every year. Therefore, the authors are interested in screening the phytochemicals in bagasse extract as well as their antioxidant activity. The obtained results would be used as basic information for further studying and adding value of this waste.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

Pure standards of ferulic acid, caffeic acid, *p*-coumaric acid, myricetin, quercetin and resveratrol were purchased from Fisher Scientific (New Jersey, USA). The standards of aescin, vanillin, gallic acid, (+)-catechins, (-)-epicatechin, rutin, compounds 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,4,6-tris(2-pyridyl)-*S*-triazine (TPTZ), Neocuproine (2,9-dimethyl-1,10-phenanthroline) and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich (USA). The organic solvents, acetic acid, methanol and acetonitrile were of HPLC grade and purchased from Merck (Darmstadt, Germany). The other chemicals and solvents used were of analytical grade and were not further purified.

### 2.2 Materials

The bagasse of 3 cultivars of sugarcane for this work was Authong 17 (AU17), Suphanburi 50 (SP50) and Suphanburi 72 (SP72). The sugarcane bagasse was dried in an oven at 60°C for 18 h, ground into small pieces and kept in a sealed bag at room temperature.

### 2.3 Preparation of crude extract

The crude extract of bagasse was extracted by methanol. Bagasse (1g) was weighed and added in a volumetric flask and then made up to 25 mL by methanol before shaking for 48 h. Each sample was extracted separately in triplicate. Solvent was then evaporated from the extracts with a rotary evaporator. The powder of extract was separated from the round bottom bottle and weighed. The exact dried weight of crude extracts was determined before adding methanol (1:10 w/v) in order to dissolve the prepared crude extract and then stored in a freezer (-20°C).

### 2.4 Total phenolic content

The total phenolic content (TPC) of the methanolic extract was determined following the method of Pastrana-Bonilla, Akoh, Sellappan, & Krewer (2003). 200 µL of the methanolic extract was mixed with 1.0 mL of 1:10 Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture solution was allowed to stand for 30 min before absorption at 765 nm was measured with a UV-Vis spectrophotometer (Cary 60, THAI UNIQUE, Thailand). Gallic acid was used as standard and results were reported as milligrams gallic acid equivalent per gram dry weight (mg GAE/g DW).

### 2.5 Total flavonoid content

The total flavonoid content (TFC) was determined following the method described by Kubola, Siriamornpun, & Meeso (2011). 500 µL of the methanolic extract was added to 200 µL of distilled water, and then 100 µL of 5% NaNO<sub>2</sub> solution was added to the mixture. 200 µL of 10% AlCl<sub>3</sub> solution was added after 6 min and then left for another 5 min before adding 500 µL of 1 M NaOH solution. After stirring and being left to stand for 15 min, the absorbance was measured by a UV-Vis spectrophotometer at 510 nm. Catechin was used as standard and results were reported as milligrams catechin equivalent per gram dry weight (mg CE/g DW).

### 2.6 Total saponin content

The total saponin content (TSC) was determined following the method of Hiai, Oura, & Nakajima (1976). Briefly, 250 µL of standard solution or methanolic extract and 250 µL of 8% vanillin-ethanol solution were mixed. 2.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> (72%) was then added to the mixture standing in an ice water bath. The mixture solution was warmed at 60°C for 15 min and then cooled to room temperature in ice-cold water. The reaction mixture was measured at 560 nm using a UV-Vis spectrophotometer against a blank. Aescin was used as standard and results were expressed as milligrams aescin equivalent per gram dry weight (mg AES/g DW).

### 2.7 Total condensed-tannins content

Total condensed-tannins content (CDT) of methanolic extracts was investigated following the modified methods of Chupin, Motillon, Charrier-El, Pizzi, & Charrier (2013). 0.5 mL of extract was mixed with 4% vanillin-methanol and 1.5 mL of 3 M HCl.

The mixture was then allowed to stand in dark at room temperature for 15 min before measuring the absorbance at 500 nm. Catechin was used as standard and results were expressed as milligrams catechin equivalent per gram dry weight (mg CE/g DW).

## 2.8 Total proanthocyanidin content

The total proanthocyanidin content (TPAC) was analyzed with the procedure of Li et al. (2006). Each 200  $\mu$ L methanolic extract solution and 1.5 mL of 4% vanillin-ethanol solution were mixed together before adding 750  $\mu$ L concentrated HCl. After 15 min, the absorbance at 500 nm was measured using a UV-Vis spectrophotometer. Catechin was used as standard and results were reported as milligrams catechin equivalent per gram dry weight (mg CE/g DW).

## 2.9 DPPH radical scavenging activity

DPPH<sup>•</sup> scavenging activity radicals of the methanolic extracts were determined according to a previously published method (Cheok, Salman, & Sulaiman, 2014). 0.5 mL of diluted methanolic extract was added to 1 mL of freshly prepared 0.1 mM DPPH in methanol solution and then incubated at room temperature in the dark for 30 min, the absorbance was detected at 517 nm using a UV-Vis spectrophotometer. The percent inhibition of the DPPH activity was calculated following equation (1).

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

Where  $A_c$  = absorbance of the control (blank) and  $A_s$  = the absorbance of the extract. The antioxidant activity represented via the 50% inhibition ( $IC_{50}$ ) value.

## 2.10 ABTS radical scavenging activity

The ABTS radical scavenging activity of the methanolic extract was determined following the method described previously (Pastrana-Bonilla Akoh, Sellappan, & Krewer, 2003). 7 mM 2,2'-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) was mixed with 2.45 mM  $K_2S_2O_8$  solution at the ratio of 1:1 to generate ABTS<sup>•+</sup> and left to stand in dark for 16 h until the reaction was completed. The absorbance of the ABTS<sup>•+</sup> solution was adjusted by distilled water to  $0.70 \pm 0.02$  at 734 nm. The reaction between 0.5 mL of the diluted methanolic extract and 1 mL of ABTS<sup>•+</sup> solution was performed with incubation at room temperature in the dark for 6 min before measuring absorbance at 734 nm using a UV-Vis spectrophotometer. The percent inhibition of

ABTS<sup>•+</sup> scavenging activity was calculated by following equation (2).

$$\text{ABTS inhibition (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (2)$$

## 2.11 Ferric reducing antioxidant power

This reducing activity of the methanolic extract was determined by the FRAP method described by Li et al. (2006). To prepare the FRAP reagent, 1.5 mL of acetate buffer (pH 3.6), 150  $\mu$ L 20 mM  $FeCl_3$  and 150  $\mu$ L 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM, HCl was mixed and warmed at 37°C. 150  $\mu$ L of methanolic extract was added to the mixture solution, and then incubated for 15 min at 37°C. The absorbance of the mixture reaction was measured at 593 nm using a UV-Vis spectrophotometer. The results were expressed as  $\mu$ mol  $Fe^{2+}$ /g DW.

## 2.12 Cupric reducing antioxidant capacity

Measurement of cupric reducing antioxidant capacity (CUPRAC) was described by Apak, Güçlü, Özyürek, & Karademir (2004). 500  $\mu$ L of  $10^{-2}$  M  $CuCl_2$  solution was mixed with 500  $\mu$ L  $7.5 \times 10^{-3}$  M neocuproine solution in ethanol and acetate buffer at pH 7.0. The methanolic extract or standard (x  $\mu$ L) and  $H_2O$  [(550 - x)  $\mu$ L] were added to the mixture solution. The absorbance was recorded at 450 nm using a UV-Vis spectrophotometer after incubation for 30 min at room temperature. The results were expressed as milligrams Trolox equivalent per gram dry weight (mg TE/g DW).

## 2.13 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 x 250 mm, i.d., 5  $\mu$ m particle size) with Shimadzu LC-20AC pumps (Shimadzu Co., Kyoto, Japan), SPD-M20A and a diode array detector. The conditions used followed Kubola, Siriamornpun, & Meeso. (2011). 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. 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, myricitin). 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.

### 2.14 Statistical analysis

All the assays were expressed as means  $\pm$  standard deviation (SD). Data analysis used the SPSS statistical software for Windows using one-way analysis of variance. The significance with  $p < 0.05$  by the Duncan test was determined. Correlations of different assays were calculated using the correlation coefficient statistical option in the Pearson test.

## 3. Results and Discussion

### 3.1 Phytochemical contents

The yields of methanolic extracts in decreasing order were SP50 ( $7.36 \pm 1.03$  %w/w) > SP72 ( $5.42 \pm$

$1.38$  % w/w) > AU17 ( $4.61 \pm 1.10$  % w/w), respectively. Table 1 shows the phytochemical content found in bagasse extracts. The results indicated that the methanolic extract of AU17 had the highest of all phytochemical contents. In AU17, TSC was the predominant substance, followed by TPC and TFC. The SP72 extract showed higher phytochemical contents than the SP50 extract, except for TSC. However, the phytochemicals found in both the SP72 and SP50 were similar amount of phytochemicals which dramatically differed from the AU17 extract. In general, phytochemicals extracted from bagasse have a lower (about 33) phytochemicals content than that found in the sugarcane (Kraphankhieo & Srihanam, 2016; Naowaset & Srihanam, 2017). However, the phytochemical content in bagasse has the same content as that found in a partially purified fraction of the sugarcane extract (Kraphankhieo & Srihanam, 2016). This was not a mistake since the types and contents of phytochemicals were varied by planted regions, climates, strain, parts of plants, harvest times, instrument analysis, solvents, methods and procedures used, diseases interface, and cultivation practices (Antoniolli, Fontana, Piccoli, & Bottini, 2015; Berli, Alonso, Bressan-Smith, & Bottini, 2012; Feng, Luo, Zhang, Zhong, & Lu, 2014; Jayaprakasha, Selvi, & Sakariah, 2003; Li, Lin, Gao, Han, & Chen, 2012).

**Table 1.** Phytochemical contents (/g DW) of methanolic extracts.

Extracts	TPC (mg GAE)	TFC (mg QE)	TSC (mg AES)	CDT (mg CE)	TPAC (mg CE)
AU17	$12.13 \pm 0.33^c$	$10.88 \pm 0.03^c$	$43.04 \pm 0.13^c$	$2.75 \pm 0.76^a$	$3.35 \pm 0.19^b$
SP50	$6.64 \pm 0.00^a$	$4.52 \pm 0.08^a$	$30.23 \pm 0.05^b$	$1.54 \pm 0.70^a$	$2.03 \pm 0.28^a$
SP72	$8.11 \pm 0.28^b$	$10.03 \pm 0.03^b$	$29.99 \pm 0.05^a$	$2.68 \pm 0.43^a$	$2.20 \pm 0.08^a$

Results are expressed as mean  $\pm$  SD of triplicate measurements. Means with different letters in the same column represent significant differences at  $p < 0.05$ . AU17, Authong 17; SP50, Suphanburi 50; SP72, Suphanburi 72; TPC, total phenolic content; TFC, total flavonoid content; TSC, total saponin content; CDT, total condensed tannin content; TPAC, total proanthocyanidin content.

### 3.2 Antioxidant activity

Antioxidants protect the body from injurious action of free radicals. No individual technique is sufficient for evaluation of antioxidant activity and various assays must be properly utilized (Frag, Abdel-Latif, Abd El Baky, & Tawfeek, 2020). Therefore, the scavenging free radicals (DPPH and ABTS assays), and metal reducing power (FRAP and CUPRAC assays) mechanism were chosen.

The antioxidant activity of the methanolic extracts is shown in Table 2. Using DPPH assay, the AU17 extract was found to have higher antioxidant activity than SP72 and SP50, respectively. However, the SP72 extract exhibited slightly higher antioxidant activity by ABTS assay than the AU17 extract. The free radical scavenging activity of all extracts had more effect on the ABTS than the DPPH radicals. The metal-reducing power activity of the AU17 extract was equally

powerful on both ferric (FRAP assay) and cupric (CUPRAC assay). In comparison with the Suphanburi cultivars, the SP72 extract showed higher metal-reducing power activity than the SP50 extract which was about 50% lower than in Au17. The tested phytochemicals showed variable antioxidation. The difference in antioxidant activity might be caused by the contents and types of phytochemicals in each bagasse cultivar (Abu Bakar, Mohamed, Rahmat, & Fry, 2009; Rice-Evans, Miller, & Paganga, 1997). Moreover, they differ vastly depending on many factors including the growing conditions, extraction process, and chemical structure (Benjakul, Kittiphattanabawon, Sumpavapol, & Maqsood, 2014; Soto-Garcia & Rosales-Castro, 2016). The flavonoids and saponin could have interacted well with  $Fe^{2+}$  via coordinate linkages (Andjelkovic et al., 2006; Moran, Klucas, Grayer, Abian, & Becana, 1997). Moreover, phenolic compounds which contain many hydroxyl groups are good antioxidants (Xia, Wu, Shi, Yang, & Zhang, 2011). These findings are in agreement with previous studies which suggested that all phenolic compounds were involved in antioxidation (Guendez, Kallithraka, Makris, & Kefalas, 2005; Katalinić et al.,

2010; Kim et al., 2006). A previous report revealed that phenolic compounds could supply H-atoms to free radicals, resulting in prevention of oxidative stress (Bendary, Francis, Ali, Sarwat, & El Hady, 2013). This stress is a major factor of various degenerative diseases (Babbar, Oberoi, & Sandhu, 2015; Farag, Abdel-Latif, Abd El Baky & Tawfeek, 2020). Zheng et al. (2017) extracted the sugarcane bagasse by various solvent systems. The hydroalcoholic extract was the best solvent to obtain the highest total phenolic and antioxidant and enzyme inhibition activities. They also concluded that sugarcane bagasse is an excellent source of natural antioxidants. This study is in agreement with Mandelli et al. (2014) who reported xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. They also suggested that the phenolic compounds are positively involved in the antioxidant activity. Moreover, Bian et al. (2013) reported that the xylooligosaccharides extracted from sugarcane bagasse also exhibited antioxidant activity, like the phenolic compounds.

**Table 2.** Antioxidant activity of methanolic extracts.

Extracts	DPPH	ABTS	FRAP	CUPRAC
	( $IC_{50}$ mg/mL)	( $IC_{50}$ mg/mL)	( $\mu$ M $Fe^{2+}$ /g DW)	(mg TE/g DW)
<b>AU17</b>	11.13 $\pm$ 0.04 <sup>a</sup>	2.49 $\pm$ 0.08 <sup>a</sup>	61.22 $\pm$ 4.61 <sup>c</sup>	5.60 $\pm$ 0.11 <sup>c</sup>
<b>SP50</b>	19.82 $\pm$ 0.09 <sup>c</sup>	3.50 $\pm$ 0.33 <sup>b</sup>	10.53 $\pm$ 1.32 <sup>a</sup>	1.19 $\pm$ 0.02 <sup>b</sup>
<b>SP72</b>	14.11 $\pm$ 0.26 <sup>b</sup>	2.39 $\pm$ 0.03 <sup>a</sup>	39.06 $\pm$ 0.76 <sup>b</sup>	3.29 $\pm$ 0.18 <sup>a</sup>

Results are expressed as mean  $\pm$  SD of triplicate measurements. Means with different letters in the same column represent significant differences at  $p < 0.05$ .

### 3.3 Correlation analysis

Previous work indicated that higher total phenolic content corresponded with stronger antioxidants activities (Zheng et al., 2017). The correlation analysis was conducted among the total phenolic, flavonoid, saponin, condensed tannin and proanthocyanidin contents and the antioxidant ability and the results are shown in Table 3. The positive correlation means that a high content of the phenolic compounds resulted from high antioxidant activity (reducing power). The positive correlations from

moderate to high value ( $r = 0.636$  to  $0.965$ ) were obtained from all tested phenolic compounds and FRAP and CUPRAC assays. In contrast, negative correlations were found for the content of the phenolic compounds and  $IC_{50}$  value (DPPH and ABTS assays). The low  $IC_{50}$  value corresponds to high antioxidant activity. The total phenolic content exhibited significant negative correlations ( $p < 0.05$ ) with DPPH ( $r = -0.900$ ), but not to ABTS ( $r = -0.618$ ). The TPC, TFC and TPAC exhibited significant negative correlations ( $p < 0.05$ ) with DPPH ( $r = -0.900$ ), while

there was no significant correlation to ABTS, except TFC ( $r = -0.953$ ). The TSC and CDT showed high negative correlation on the DPPH assay ( $r = -0.752, -0.697$ ). The CDT showed high negative correlation on ABTS ( $r = -0.755$ ), but TPC and TPAC showed the moderate negative correlation ( $r = -0.618, -0.514$ ). In addition, significant correlations ( $r > 0.900, p < 0.05$ ) among the four antioxidant methods were also found, except for TPC with the ABTS method. The results strongly indicated that the substances in sugarcane

bagasse are chiefly responsible for its antioxidant property. The conclusion is in agreement with previous reports (Sun, Chu, Wu, & Liu 2002; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006; Wojdylo, Oszmianski, & Czemerys, 2007; Zheng et al., 2017). Thus plants with rich phenolic content can be a valuable source of antioxidants.

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

Factors	TPC	TFC	TSC	TPAC	CDT	DPPH	ABTS	FRAP	CUPRAC
TPC	1	.785*	.958**	.945**	.555	-.900**	-.618	.939**	.965**
TFC		1	.589	.667*	.726*	-.975**	-.953**	.940**	.908**
TSC			1	.954**	.383	-.752*	-.393	.815**	.871**
TPAC				1	.400	-.806**	-.514	.848**	.896**
CDT					1	-.697*	-.755*	.650	.636
DPPH						1	.880**	-.988**	-.977**
ABTS							1	-.823**	-.779*
FRAP								1	.991**
CUPRAC									1

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

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

### 3.4 Identification and quantification of phenolic compounds

The individual phenolic contents in the methanolic extracts are presented in Table 4. The main phenolic compounds; caffeic acid, catechin, epicatechin, gallic acid, ferulic acid, myricetin,

quercetin, *p*-coumaric acid, resveratrol, and rutin were investigated. The results revealed that the AU17 extract had the highest concentrations of phenolic compounds, following by the SP72 and SP50, respectively. Gallic acid and *p*-coumaric acid were the predominant phenolics in the AU17 extracts while gallic acid, caffeic acid, and *p*-coumaric acid were predominant phenolics in the SP72 extract. Surprisingly, two types of phenolics; gallic acid, and *p*-coumaric acid were not found in the SP50 extract. All methanolic extracts had a similar content of ferulic acid. Proanthocyanidins in the form of monomeric phenolic compounds, such as epicatechin, were the major substances in all extracts. However, catechin was not found in both Au17 and SP50 but was observed in the SP72 extract. In addition, rutin was not found in the SP50. Myricetin was found in all extracts with low content as in the case of rutin. The large content of phenolic compounds found in the methanolic extracts were in agreement with previous

reports (Perumalla & Hettiarachchy, 2011). There has been interest in the further use of mono flavonoids due to their pharmacological effect (Guendez, Kallithraka, Makris, & Kefalas, 2005). Besides phytochemical compounds, flavonols (myricetin) were found in low content and this also agreed with previous reports (Burin, Ferreira-Lima, Panceri, & Bordignon-Luiz, 2014). It was previously reported that resveratrol is found in generally low content only in fruit pulp (Yilmaz & Toledo, 2004). This was in contrast to this work due to the resveratrol being found in moderate contents in the methanolic extracts of AU17 and was equal to quercetin content in this same cultivar. Moreover, resveratrol was also found in the methanolic extracts of SP50 and SP72 in equal amounts. The results suggested that both types and contents of phytochemicals were influenced by cultivars of sugarcane.

**Table 4.** Composition and content of phenolic compound (mg/g DW) in methanolic extracts.

Phenolic compounds	AU17	SP50	SP72
Gallic acid	1.498 ± 0.002 <sup>b</sup>	ND	0.472 ± 0.017 <sup>a</sup>
Caffeic acid	0.557 ± 0.002 <sup>c</sup>	ND	0.547 ± 0.001 <sup>b</sup>
<i>p</i> -Coumaric acid	0.960 ± 0.035 <sup>c</sup>	0.460 ± 0.036 <sup>a</sup>	0.543 ± 0.034 <sup>b</sup>
Ferulic acid	0.323 ± 0.001 <sup>c</sup>	0.317 ± 0.003 <sup>b</sup>	0.306 ± 0.001 <sup>a</sup>
Resveratrol	0.432 ± 0.024 <sup>b</sup>	0.213 ± 0.006 <sup>a</sup>	0.246 ± 0.014 <sup>a</sup>
Catechin	ND	ND	0.031 ± 0.011 <sup>b</sup>
Epicatechin	0.232 ± 0.019 <sup>b</sup>	0.156 ± 0.003 <sup>a</sup>	0.158 ± 0.023 <sup>a</sup>
Quercetin	0.430 ± 0.015 <sup>a</sup>	0.464 ± 0.031 <sup>a</sup>	0.454 ± 0.005 <sup>a</sup>
Rutin	0.014 ± 0.001 <sup>b</sup>	ND	0.001 ± 0.003 <sup>a</sup>
Myricetin	0.006 ± 0.002 <sup>a</sup>	0.015 ± 0.004 <sup>b</sup>	0.018 ± 0.002 <sup>b</sup>

Results are expressed as mean ± SD of triplicate measurements. Means with different letters in the same column represent significant differences at  $p < 0.05$ . ND = not detected

#### 4. Conclusion

This work was focused on phytochemicals and antioxidant activity of methanolic extracts in bagasse from different cultivars. In general, the phytochemicals and antioxidant activity varied by the cultivar of bagasse sugarcane. The methanolic extract of the AU17 showed the highest total phytochemicals content and antioxidant activity. The phytochemical

contents found in the bagasse extracts were positively related to their antioxidant potential. The HPLC data showed that all studied bagasse cultivars contained various phenolic compounds and also strong antioxidant potential. This work suggested that bagasse is a good source of health- supplement compounds, promising antioxidant activity via free radicals scavenging and reducing power mechanisms. It would be interesting to focus more studies on the

biological activities of their individual purified extracts.

### 5. Acknowledgements

This Research was Financially Supported by Mahasarakham University (Grant year 2020). The authors also thank Department of Chemistry, Faculty of Science, Mahasarakham University for chemical and reagent support.

### 6. References

- Abu Bakar, M. F., Mohamed, M., Rahmat, A., & Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). *Food Chemistry*, *113*, 479-483. doi:10.1016/j.foodchem.2008.07.081
- Alghazeer, R., El-Saltani, H., Saleh, N., Al-Najjar, A., & Hebail, F. (2012). Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. *Natural Science*, *4*, 324-335. doi:10.4236/ns.2012.45045
- Andjelkovic, M., Camp, J. V., Meulenaer, B. D., Depaemelaere, G., Socaciu, C., Verloo, M., & Verhe, R. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chemistry*, *98*(1), 23-31. doi:10.1016/j.foodchem.2005.05.044
- Antoniolli, A., Fontana, A. R., Piccoli, P., & Bottini, R. (2015). Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. *Food Chemistry*, *178*, 172-178. doi:10.1016/j.foodchem.2015.01.082
- Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, *52*(26), 7970-7981. doi:10.1021/jf048741x
- Babbar, N., Oberoi, H. S., & Sandhu, S. K. (2015). Therapeutic and nutraceutical potential of bioactive compounds extracted from fruit residues. *Critical Reviews in Food Science and Nutrition*, *55*(3), 319-337. doi:10.1080/10408398.2011.653734
- Bendary, E., Francis, R. R., Ali, H. M. G., Sarwat, M. I., & El Hady, S. (2013). Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. *Annals of Agricultural Science*, *58*(2), 173-181. doi:10.1016/j.aos.2013.07.002
- Benjakul, S., Kittiphattanabawon, P., Sumpavapol, P., & Maqsood, S. (2014). Antioxidant activities of lead (*Leucaena leucocephala*) seed as affected by extraction solvent, prior dechlorophyllisation and drying methods. *Journal of Food Science and Technology*, *51*(11), 3026-3037. doi:10.1007/s13197-012-0846-1
- Berli, F. J., Alonso, R., Bressan-Smith, R., & Bottini, R. (2012). UV-B impairs growth and gas exchange in grapevines grown in high altitude. *Physiologia Plantarum*, *149*(1), 127-140. doi:10.1111/ppl.12012
- Bian, J., Peng, F., Peng, X.-P., Peng, P., Xu, F., & Sun, R.-C. (2013). Structural features and antioxidant activity of xylooligosaccharides enzymatically produced from sugarcane bagasse. *Bioresource Technology*, *127*, 236-241. doi:10.1016/j.biortech.2012.09.112
- Burin, V. M., Ferreira-Lima, N. E., Panceri, C. P., & Bordignon-Luiz, M. T. (2014). Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: Evaluation of different extraction methods. *Microchemical Journal*, *114*, 155-163. doi:10.1016/j.microc.2013.12.014
- Butsat, S., & Siriamornpun, S. (2010). Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chemistry*, *119*(2), 606-613. doi:10.1016/j.foodchem.2009.07.001
- Butsat, S., Weerapreeyakul, N., & Siriamornpun, S. (2009). Changes in phenolic acids and antioxidant activity in Thai rice husk at five growth stages during grain development. *Journal of Agricultural and Food Chemistry*, *57*, 4566-4571. doi:10.1021/jf9000549
- Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, *51*, 15-25. doi:10.1016/j.fct.2012.09.021
- Cheok, C. Y., Salman, H. A. K., & Sulaiman, R. (2014). Extraction and quantification of saponins: A review. *Food Research International*, *59*, 16-40. doi:10.1016/j.foodres.2014.01.057
- Chupin, L., Motillon, C., Charrier-El, B. F., Pizzi, A., & Charrier, B. (2013). Characterisation of maritime pine (*Pinus pinaster*) bark tannins extracted under different conditions by

- spectroscopic methods, FTIR and HPLC. *Industrial Crops and Products*, 49, 897-903.  
doi:10.1016/j.indcrop.2013.06.045
- Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., & Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clinical Chemistry*, 52(4), 601-623.  
doi:10.1373/clinchem.2005.061408
- Denev, P. N., Kratchanov, C. G., Ciz, M., Lojek, A., & Kratchanova, M. G. (2012). Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: *in vitro* and *in vivo* evidences and possible mechanisms of action: A review. *Comprehensive Reviews in Food Science and Food Safety*, 11(5), 471-489.  
doi:10.1111/j.1541-4337.2012.00198.x
- Duarte-Almeida, J. M., Negri, G., Salatino, A., de Carvalho, J. E., & Lajolo, F. M. (2007). Antiproliferative and antioxidant activities of a tricin acylated glycoside from sugarcane (*Saccharum officinarum* L.) juice. *Phytochemistry*, 68(8), 1165-1171.  
doi:10.1016/j.phytochem.2007.01.015
- Farag, R. S., Abdel-Latif, M. S., Abd El Baky, H. H., & Tawfeek, L. S. (2020). Phytochemical screening and antioxidant activity of some medicinal plants' crude juices. *Biotechnology Reports*, 28, e00536.  
doi:10.1016/j.btre.2020.e00536
- Feng, S., Luo, Z., Zhang, Y., Zhong, Z., & Lu, B. (2014). Phytochemical contents and antioxidant capacities of different parts of two sugarcanes (*Saccharum officinarum* L.) cultivars. *Food Chemistry*, 151, 452-458.  
doi:10.1016/j.foodchem.2013.11.057
- Guendez, R., Kallithraka, S., Makris, D. P., & Kefalas, P. (2005). Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seed extracts: Correlation with antiradical activity. *Food Chemistry*, 89(1), 1-9.  
doi:10.1016/j.foodchem.2004.02.010
- Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some saponinins and saponins with vanillin and sulfuric acid. *Planta Medica*, 29(2), 116-122. doi:10.1055/s-0028-1097639
- Jayaprakasha, G. K., Selvi, T., & Sakariah, K. K. (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International*, 36(2), 117-122.  
doi:10.1016/S0963-9969(02)00116-3
- Katalinić, V., Možina, S. S., Skroza, D., Generalić, I., Abramović, H., Miloš, M., ... Boban, M. (2010). Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). *Food Chemistry*, 119(2), 715-723.  
doi:10.1016/j.foodchem.2009.07.019
- Kim, S.-Y., Jeong, S.-M., Park, W.-P., Nam, K. C., Ahn, D. U., & Lee, S.-C. (2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. *Food Chemistry*, 97(3), 472-479.  
doi:10.1016/j.foodchem.2005.05.027
- Kraphankhieo, W., & Srihanam, P. (2016). Investigation of phytochemical and antioxidant activity of different parts of sugarcane planted in Buriram province. *Proceedings of the 12th Mahasarakham University Research Conference* (pp. 728-743). Retrieved from [http://journal.msu.ac.th/eng/index.php?page=show\\_journal\\_article&j\\_id=5&article\\_id=1632](http://journal.msu.ac.th/eng/index.php?page=show_journal_article&j_id=5&article_id=1632)
- Kubola, J., Siriamornpun, S., & Meeso, N. (2011). Phytochemicals, vitamin C and sugar content of Thai wild fruits. *Food Chemistry*, 126, 972-981.  
doi:10.1016/j.foodchem.2010.11.104
- Li, X., Lin, J., Gao, Y., Han, W., & Chen, D. (2012). Antioxidant activity and mechanism of *Rhizoma Cimicifugae*. *Chemistry Central Journal*, 6(1), 1-10. doi:10.1186/1752-153X-6-140
- Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96(2), 254-260.  
doi:10.1016/j.foodchem.2005.02.033
- Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8), 118-126.  
doi:10.4103/0973-7847.70902
- Mandelli, F., Brenelli, L. B., Almeida, R. F., Goldbeck, R., Wolf, L. D., Hoffmann, Z. B., ... Squina, F. M. (2014). Simultaneous production of xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. *Industrial Crops and Products*, 52, 770-775.  
doi:10.1016/j.indcrop.2013.12.005
- Meng, J. F., Fang, Y. L., Qin, M. Y., Zhuang, X. F., & Zhang, Z. W. (2012). Varietal differences among the phenolic profiles and antioxidant properties of four cultivars of spine grape (*Vitis davidii* Foex) in Chongyi County (China). *Food Chemistry*, 134(4), 2049-2056.  
doi:10.1016/j.foodchem.2012.04.005

- Moran, J. F., Klucas, R. V., Grayer, R. J., Abian, J., & Becana, M. (1997). Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: Prooxidant and antioxidant properties. *Free Radical Biology and Medicine*, 22, 861-870. doi:10.1016/s0891-5849(96)00426-1
- Naowaset, D., & Srihanam, P. (2017). Phytochemical contents and antioxidant activity of partially purified sugarcane extract by silica gel column. *Proceedings of the 13th Mahasarakham University Research Conference* (pp. 444-453). Retrieved from [http://www.journal.msu.ac.th/index.php?page=show\\_journal\\_article&j\\_id=5&article\\_id=2018](http://www.journal.msu.ac.th/index.php?page=show_journal_article&j_id=5&article_id=2018)
- Pastrana-Bonilla, E., Akoh, C. C., Sellappan, S., & Krewer, G. (2003). Phenolic content and antioxidant capacity of muscadine grapes. *Journal of Agricultural and Food Chemistry*, 51(18), 5497-5503. doi:10.1021/jf030113c
- Perumalla, A. V. S., & Hettiarachchy, N. S. (2011). Green tea and grape seed extracts - Potential applications in food safety and quality. *Food Research International*, 44(4), 827-839. doi:10.1016/j.foodres.2011.01.022
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends of Plant Science*, 2(4), 152-159. doi:10.1016/S1360-1385(97)01018-2
- Soto-Garcia, M., & Rosales-Castro, M. (2016). Effect of solvent and solvent-to-solid ratio on the phenolic extraction and the antioxidant capacity of extracts from *Pinus durangensis* and *Quercus sideroxylla* bark. *Maderas: Ciencia y Tecnologia*, 18(4), 701-714. doi:10.4067/S0718-221X2016005000061
- Sun, J., Chu, Y., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry*, 50, 7449-7454. doi:10.1021/jf0207530
- Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19, 669-675. doi:10.1016/j.jfca.2006.01.003
- Wojdylo, A., Oszmianski, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105, 940-949. doi:10.1016/j.foodchem.2007.04.038
- Xia, D., Wu, X., Shi, J., Yang, Q., & Zhang, Y. (2011). Phenolic compounds from the edible seeds extract of Chinese Mei (*Prunus mume* Sieb. et Zucc) and their antimicrobial activity. *LWT - Food Science and Technology*, 44(1), 347-349. doi:10.1016/j.lwt.2010.05.017
- Yilmaz, Y., & Toledo, R. T. (2004). Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *Journal of Agricultural and Food Chemistry*, 52(2), 255-260. doi:10.1021/jf030117h
- Zheng, R., Su, S., Zhou, H., Yan, H., Ye, J., Zhao, Z., You, L., & Fu, X. (2017). Antioxidant/antihyperglycemic activity of phenolics from sugarcane (*Saccharum officinarum* L.) bagasse and identification by UHPLC- HR- TOFMS. *Industrial Crops and Products*, 101, 104-114. doi:10.1016/j.indcrop.2017.03.012