OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2020.XX.XX

Research Article
Oxidative Compounds Screening in the Extracts of Sugarcane (*Saccharum officinarum* L.) Planted in Maha Sarakham Province, Thailand

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Abstract

Background and Objective: Oxidative compounds are gradually interested and studied, especially agricultural sources for value-added in health and cosmetics. The objective of the present study was to screen the oxidative compounds of the sugarcane (*Saccharum officinarum* L.) planted in Maha Sarakham Province, Thailand. **Materials and Methods:** The four cultivars of sugarcane were extracted by ethanol before the screening of phytochemicals; total phenolic, flavonoid, triterpenoid, saponin and condensed-tannin. The extracts were then tested for antioxidation using different assays. Correlation between the phytochemical and antioxidation as well as the individual phenolic compounds were analyzed. **Results:** All tested phytochemicals found in the node extracts higher content than the rind extracts. The node extracts of all sugarcane cultivars have a higher potent activity than the rind extracts. The phenolic compounds and antioxidation activity were significantly different by cultivars and parts of the sugarcane. All phytochemicals have positively correlated to all tested methods with moderate to high values. The main substances were catechin, quercetin, ferulic acid, resveratrol, gallic acid and epicatechin respectively. **Conclusion:** The sugarcane is an important source of phytochemicals which expressed high antioxidant activity. It might be used the phytochemicals from the sugarcane for health and beauty applications.

Key words: Extraction, cultivar, phytochemicals, sugarcane, antioxidation

Citation: Kerdchan, K., N. Kotsaeng and P. Srihanam, 2020. Oxidative compounds screening in the extracts of sugarcane (*Saccharum officinarum* L.) Planted in Maha Sarakham Province, Thailand. Asian J. Plant Sci., 19: XX-XX.

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

In present, humans getting harm from free radicals (oxidants), a group of inactive molecules composed of the lone-pair electron. Overproduction of the free radicals leading to so-called oxidative stress, which could be induced some degenerative diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, neurological disorders, aging and other degenerative diseases in humans are affected by the oxidative stress[1,2]. Therefore, the antioxidant activity of the phytochemicals is one of the interesting topics which popularly studied and reported[3-8]. The substances derived from plants called "phytochemicals" including phenolics, flavonoids, quinines, tannins, alkaloids, saponins and sterols have been gradually studied from the past ten years ago[9-12]. The phytochemicals are secondary metabolites produced for specific proposes such as defense mechanism, color and smelt. They have been applied as folk medicinal and in nutritional applications from the past until now[13,14]. This was according to they have various biological activities such as antioxidant, antibacterial, anti-inflammatory, anti-diabetic and anti-aging[15-17]. The substances obtained from plants have also proven their safety without side effects compared with synthetic substances. However, sources are critical factors in obtaining desired phytochemicals. On the other hand, various plants have not been discovered and lack of bioactive compounds information[18-22].

Sugarcane (*Saccharum officinarum* L.) is a main economic crop of many countries includes Thailand. The main application of sugarcane is sugar production. Sugarcane was also used for ethanol production as fuel instead of petroleum source[23]. Moreover, some reports about phytochemicals in sugarcane have been discovered[24-27]. In Thailand, sugarcane is planted in many places, especially in the East and North-Eastern area. They are used as raw materials for sugar production. Maha Sarakham is a central land of North-Eastern Thailand. This province has a factory for sugar production. There are many cultivars of sugarcane planted in this province. However, the information about phytochemicals and their antioxidation has not been studied. Therefore, the goals of this work were to extract and screen some phytochemicals and their antioxidant activity in different cultivars of sugarcane planted in Maha Sarakham, province Thailand.

MATERIALS AND METHODS

Study area: This work was done for six months from December 1, 2019-May 30, 2020. The experiment was performed at the Faculty of Pharmacy, Maha Sarakham University, Thailand.

Materials: The four cultivars of sugarcane, Au-thong 15, KK3, KK80 and KK07-037 were kindly supplied from Agricultural Research and Development Center Mahasarakham, Maha Sarakham, Thailand. The sugarcane samples were separated for node and rind parts and 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.

Crude extraction: The 15 g of sugarcane powder was immersed in a 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 the solvent by rotary evaporator. The dried crude extracts were dissolved by ethanol and stored in a freezer until analysis.

Total phenolic content: The Total Phenolic Content (TPC) of the ethanolic extract was determined[28]. The ethanolic 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[29]. The ethanolic extracts were mixed with distilled water, 5% $NaNO_2$ solution and 10% $AlCl_3$ solution. Finally, 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 (TSaC) was determined[30]. The ethanolic 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 triterpenoid content: The Total Triterpenoid Content (TTC) was determined[31].The ethanolic extracts were heated to dryness in a water bath at 99°C. Then the mixture of a vanillin-acetic acid solution (5:95, w/v) and 0.8 mL perchloric acid was added and incubated at room temperature for 15 min. Finally, acetic acid was added and left for 15 min before measuring the absorbance at 548 nm. The ursolic acid was used as a standard.

Total condensed-tannin content: Total condensed-tannin content (CDT) was investigated following the previously studied method[32].The ethanolic 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 ethanolic extracts was determined according to a previously published method[33]. 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[28]. A 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was mixed with $K_2S_2O_8$ solution to generate $ABTS^{\cdot+}$ and the absorbance at 734 nm was adjusted by distilled water to 0.700±0.020. The ethanolic extracts were mixed with $ABTS^{\cdot+}$ solution in the dark for 6 min before measuring at 734 nm. The percent inhibition of $ABTS^{\cdot+}$ 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 ethanolic extracts was determined by FRAP method[34]. 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 mM HCl)was mixed with the ethanolic extract and then incubated for 15 min at 37 °C. The absorbance at 593 nm was measured and expressed results as mmol Fe^{2+}/g DW.

Identification and quantification of phenolic compounds: The RP-HPLC analysis with Shimadzu LC-20AC pumps (Shimadzu Co., Kyoto, Japan), SPD-M20A with a diode array detector and chromatographic separations were performed on a column Inertsil ODS-3, C18 (4.6×250 mm, i.e. 5 µm). The conditions used were followed by previously reported method[29]. The mobile phase was adjusted at a flow rate of 0.8 mL min^{-1}. The elution was performed by the gradient system and a re-equilibration period of 5 min between individual runs. The column temperature of 38°C and 20 µL injection volume were maintained. The spectra were recorded from 200-600 nm depending on each standard. Phenolic compounds were identified by comparing with those of external standard compounds.

Statistical analysis: The mean+standard deviation (SD) and Duncan's new multiple range test was used to evaluate the significant differences with $p<0.05$ and $p<0.01$. Pearson's correlation coefficient (r) was used to indicate data correlation.

RESULTS

Oxidative compounds: Table 1 shows phytochemical contents found in sugarcane extracts. The results indicated that all tested substances were varied by parts and cultivars of the sugarcane. In general, the content of all phytochemicals found higher in the node extract. Among the tested oxidative compounds, the TPC found the highest in KK07-037 which slightly higher than KK3 and Au-thong 15 cultivars. The TFC of the extracts had lower content than the TPC and the Au-thong 15 cultivars showed the highest value. The TTC found the lowest content comparing to other compounds. The KK80 cultivar showed the highest content of TTC. In this study, the

Table 1: Oxidative compounds in the extracts of different sugarcane cultivars

Cultivars	TFC (mg CE/g DW)	TPC (mg GAE/g DW)	TTC (mg UR/g DW)	TSaC (mg AES/g DW)	CDT (mg CE/g DW)
Au-thong 15 rind	5.19±0.24^{c}	6.17±0.20^{bc}	0.14±0.01^{e}	67.48±3.08^{c}	32.93±0.86^{d}
node	6.60±0.15^{a}	6.80±0.31^{a}	0.18±0.00^{bc}	86.96±7.42^{a}	37.65±2.01^{c}
KK 3 rind	4.64±0.31^{d}	6.17±0.43^{bc}	0.16±0.00^{d}	82.40±4.58^{ab}	33.14±1.48^{d}
node	5.94±0.33^{b}	6.90±0.23^{a}	0.17±0.01^{c}	88.15±2.93^{a}	36.75±0.31^{c}
KK 80 rind	5.18±0.20^{c}	5.79±0.08^{c}	0.19±0.01^{ab}	76.86±7.33^{b}	49.11±0.49^{b}
node	5.29±0.07^{c}	6.37±0.19^{b}	0.20±0.01^{a}	76.96±0.54^{b}	55.00±2.98^{a}
KK 07-037 rind	4.70±0.06^{d}	5.35±0.12^{d}	0.13±0.01^{e}	62.40±1.92^{c}	33.66±1.18^{d}
node	5.28±0.12^{c}	7.07±0.16^{a}	0.17±0.01^{c}	76.45±4.76^{b}	35.31±0.70^{cd}

Results are expressed as Mean±SD of triplicate measurements, Means with different letters in the same column represent significant differences at $p<0.05$, TFC: Total flavonoid content, TPC: Total phenolic content, TTC: Total triterpenoid content, TSaC: Total saponin content, CDT: Total condensed-tannin content, CE: Catechin equivalent, DW: Dried weight, GAE: Gallic acid equivalent, UR: Ursolic acid equivalent, AES: Aescin equivalent, g: gram

TSaC found the highest content between the tested oxidative compounds. Almost the cultivars found the TSaC in the node extracts higher than rind, except the KK80 cultivar found the TSaC in the rind extract than node. Among cultivars, the KK3 had the highest content of TSaC. The CDT found high content followed the TSaC. The highest content of the CDT found in the KK80 extract.

Antioxidation screening: Table 2 showed antioxidation results expressed by IC_{50} values. This value is an inhibitory concentration of the extract using for scavenging 50% of the free radicals. The low IC_{50} values indicated high potential antioxidation of the extract. In general, the node extracts of all sugarcane cultivars have lower IC_{50} value than the rind extracts. With DPPH assay, the Au-thong 15 showed slightly lower IC_{50} than other cultivars. Therefore, the extracts of Au-thong 15 have higher antioxidation than others. ABTS assay showed that the rind extracts have higher IC_{50} values than the node extracts in all cultivars. This result indicated that the rind extracts have higher antioxidation than the node extracts. The KK80 showed the highest activity for than others. FRAP assay showed similar trend with the scavenging method and the node extract of KK3 cultivar had the highest activity.

Correlation analysis: Table 3 showed a correlation between the oxidative compounds and the antioxidation of the sugarcane. The results indicated that all tested phytochemicals, TPC, TFC, TSaC, TTC and CDT have a moderate positive correlation together. The TFC had a high positive correlation to the TSaC while the TSaC showed a high positive correlation to other compounds. Considering to the antioxidation, the TFC had a high positive correlation to DPPH and FRAP assays but had a moderate correlation with ABTS assay. The TPC had a high positive correlation to both DPPH and ABTS assays but had a moderate correlation with FRAP. The TTC did not involve in antioxidation to DPPH but had a high positive correlation to ABTS and FRAP assays. The CDT revealed a similar correlation like TTC. The TSaC showed correlation to radical scavenging methods in low level but had a moderate correlation with FRAP.

Table 2: Antioxidation screening of the sugarcane extracts

	Methods		
Cultivars	DPPH (IC_{50} mg mL^{-1})	ABTS (IC_{50} mg mL^{-1})	FRAP (uM $FeSO_4$/g DW)
Au-thong 15			
Rind	0.57 ± 0.06^{c}	13.34 ± 0.22^{b}	101.15 ± 9.23^{c}
Node	0.44 ± 0.01^{e}	11.09 ± 0.22^{c}	127.47 ± 4.73^{b}
KK 3			
Rind	0.74 ± 0.02^{a}	16.05 ± 0.38^{a}	74.31 ± 3.39^{d}
Node	0.55 ± 0.01^{d}	10.37 ± 0.35^{d}	143.69 ± 3.69^{a}
KK 80			
Rind	0.76 ± 0.02^{a}	13.67 ± 0.18^{b}	106.18 ± 3.12^{c}
Node	0.57 ± 0.01^{cd}	7.31 ± 0.18^{e}	127.46 ± 6.46^{b}
KK 07-037			
Rind	0.64 ± 0.01^{b}	13.36 ± 0.12^{b}	106.06 ± 4.22^{c}
Node	0.57 ± 0.01^{cd}	10.11 ± 0.13^{d}	109.19 ± 3.54^{c}

Results are expressed as Mean±SD of triplicate measurements, Means with different letters in the same column represent significant differences at $p<0.01$, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2′-azino-bis(3-ethylbenothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power, IC_{50}: Half minimum inhibitory concentration, Mg: Milligram, Ml: Milliliter, Um: Micro molar, G: Gram, DW: Dried weight, KK: Khon kae

Table 3: Correlation coefficient(*r*) between the phytochemicals and antioxidation activity

	TFC	TPC	TTC	TSaC	CDT	DPPH	ABTS	FRAP
TFC	1	0.581**	0.431*	0.575**	0.071	-0.755**	-0.455*	0.692**
TPC		1	0.429*	0.648**	0.074	-0.581**	-0.521**	0.423*
TTC			1	0.581**	0.768**	-0.090	-0.558**	0.427*
TSaC				1	0.113	-0.240	-0.205	0.350
CDT					1	-0.097	-0.533**	0.347
DPPH						1	0.644**	0.668**
ABTS							1	0.767**
FRAP								1

**Correlation is significant at the $p<0.01$, TFC: Total flavonoid content, TPC: Total phenolic content, TTC: Total triterpenoid content, TSaC: Total saponin content, CDT: Total condensed-tannin content, DPPH (2,2-diphenyl-1-picrylhydrazyl); ABTS: 2,2′-azino-bis(3-ethylbenothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power

Table 4: Types and contents(mg/g DW) of oxidative compounds analysis by HPLC

Cultivars	Gallic acid	Catechin	Caffeic acid	Epicatechin	p-Coumaric acid	Ferulic acid
Au-thong 15						
Rind	1.35±0.15^{d}	8.10±0.57^{e}	0.35±0.01^{c}	2.08±0.16^{b}	0.11±0.01^{c}	5.06±0.54^{c}
node	1.30±0.12^{d}	7.54±1.15^{e}	0.41±0.25^{a}	1.93±0.16^{b}	0.13±0.02^{c}	3.80±0.48^{d}
KK 3						
Rind	2.39±0.26^{b}	84.75±6.45^{a}	0.33±0.01^{c}	1.54±0.16^{c}	0.18±0.00^{b}	9.76±1.27^{a}
Node	1.27±0.07^{d}	43.78±3.46^{b}	0.34±0.00^{c}	1.22±0.07^{d}	0.21±0.01^{a}	10.61±0.03^{a}
KK 80						
Rind	2.19±0.11^{b}	49.40±0.85^{c}	0.38±0.00^{b}	1.94±0.10^{b}	0.10±0.01^{d}	7.03±0.40^{b}
Node	3.23±0.41^{a}	23.60±2.14^{d}	0.37±0.00^{b}	0.82±0.14^{e}	0.09±0.00^{d}	3.40±0.32^{d}
KK07-037						
Rind	1.34±0.09^{d}	19.55±2.48^{d}	0.34±0.01^{c}	2.65±0.14^{a}	0.19±0.02^{b}	6.56±0.64^{b}
Node	1.75±0.07^{c}	10.82±0.45^{e}	0.37±0.02^{b}	1.48±0.06^{c}	0.13±0.01^{c}	3.40±0.20^{d}

Cultivars	Rutin	Myricetin	Resveratrol	Quercetin
Au-thong 15				
Rind	0.24±0.02^{c}	0.46±0.05^{bc}	5.64±1.00^{c}	10.47±0.54^{d}
Node	0.24±0.01^{c}	0.31±0.09^{c}	6.74±1.36^{bc}	11.40±1.25^{d}
KK 3				
Rind	0.57±0.09^{a}	1.16±0.22^{a}	8.61±0.65^{a}	25.52±2.03^{b}
Node	0.49±0.07^{b}	1.11±0.08^{a}	8.39±1.22^{ab}	30.51±2.62^{a}
KK 80				
Rind	0.29±0.02^{c}	0.49±0.03^{b}	5.27±0.03^{c}	14.74±0.49c
Node	0.10±0.00^{d}	0.04±0.00^{d}	6.59±0.68b^{c}	5.02±0.43^{e}
KK07-037				
Rind	0.28±0.01^{c}	0.58±0.06^{b}	6.64±1.07^{bc}	23.87±1.57^{b}
Node	0.11±0.03^{d}	0.14±0.04^{d}	7.46±0.97^{ab}	6.29±0.54^{e}

Results are expressed as Mean±SD of triplicate measurements, Means with different letters in the same column represent significant differences at p<0.01, mg: milligram, g: gram, DW: Dried weight, HPLC: High-Performance liquid chromatography, KK: Khon kaen

Phenolic compounds analysis: The types and contents of the phenolic compounds showed in Table 4. Generally, the phenolic compounds varied by the cultivars and parts of sugarcane. Au-thong 15 had similar contents of the compound's comparison between the rind and node extracts. The dominant substances were quercetin, catechin, resveratrol and epicatechin. The predominant of phenolic acid was ferulic acid and gallic acid. The KK3 had the highest content of the oxidative compounds followed by the KK80 except for gallic acid. The dominant substances in both cultivars were similar type and sequence as found in the Au-thong 15. The KK07-037 showed similarly but had slightly higher contents of oxidative compounds than Au-thong 15.

DISCUSSION

The phenolic compounds, the secondary metabolite of plants have been reported for various biological activities[35-38]. The results obtained from this work showed that the node extracts have higher phytochemical contents than in the rind extracts. This was controversy comparing to previously reported[31] that the rind of sugarcane composed the highest phytochemicals than other parts. This contrast results were in agreed when compared the flavonoid found in the pulp of sugar-beet, tomato and sesame[39]. The secondary metabolite found generally in plants. However, the types and contents were varied by cultivars and parts[31]. Furthermore, this variability was influenced by other factors such as geography, climate, harvesting time, season, methods and instrument for analysis[40-42].

It is well known that one method is not enough for screening the antioxidation of the phytochemicals[44]. This was due to the complexation of each substance. The antioxidation activity of the sugarcane extracts was tested by free radical scavenging assays (DPPH and ABTS). The antioxidative compounds could be transferred H-atom to DPPH· or electron to $ABTS^{\cdot+}$ to form stable molecules[43,44]. The obtained results suggested that the sugarcane planted in Maha Sarakham, Thailand had higher antioxidation than China sugarcane[31]. The Ferric Reducing Antioxidant Power (FRAP) is of simple, fast and inexpensive method for antioxidation testing. This assay measured the reducing oxidation number of Fe^{3+} (ferric) into Fe^{2+} (ferrous)[45]. The reducing power of the sugarcane extract obtained in this work was lower than grape seed extract[46]. The previous report suggested that the position of leaving groups like hydroxyl (-OH), ortho-dihydroxyl and an adjacent double bond in carbon ring affected to the antioxidation activity of the compounds[9,47,48].

The large contents of phenolic compounds found in the sugarcane extracts were in agreement with previous reported,

especially catechin and epicatechin[49]. The mono flavonoids have been interested in further use due to their pharmacological effect[50]. Besides phytochemical compounds, flavonols (myricetin) were found in low content. This also agreed with the previous reports[51]. Among oxidative compounds, flavonoids found the highest contents which similar related to previous reports[52,53]. Previously reported that resveratrol found generally low content only in fruit pulp[28,54]. It was a contrast in this work due to the resveratrol was found similar contents in rind and node extracts. The obtained results suggested that both types and contents of phytochemicals affected by cultivars and parts of sugarcane[31]. In the future study, different biological activities such as antibacterial, enzyme inhibition effect and antidiabetic would be performed to obtain more information of the finding compounds.

CONCLUSION

The node extracts of four cultivars of sugarcane planted in Maha Sarakham, Thailand showed higher phytochemical contents than the rind. The phenolic compounds as well as the antioxidation varied by the cultivars and the parts of sugarcane. The antioxidation activity had a moderate to high positive correlation to each phytochemical. Based on HPLC analysis, the large contents found in sugarcane were flavonoids such as quercetin, catechin, resveratrol and ferulic acid which higher many folds than phenolic acid-like gallic acid and *p*-coumaric acid.

SIGNIFICANCE STATEMENT

This study screening the oxidative compounds and individual phenolic compounds of the four cultivars sugarcane extracts; planted in Maha Sarakham, Thailand that can be beneficial for further application as health supplement substances or a 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 oxidative compounds of sugarcane may be arrived at.

ACKNOWLEDGMENT

This research financially supported by Mahasarakham University (Grant year 2020). We would like to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Thailand, for partial financial support.

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