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# Fractionation of Sugarcane Extracts and Determination of Phenolic Compounds and their Antioxidant Activity

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Crude ethanolic extracts of four cultivars of sugarcane (*Saccharum officinarum* L.) were firstly prepared and then fractionated using silica gel column chromatography before the determination of their oxidative substances. The fractionated extracts were then tested for antioxidant activity by various assays. Finally, high-performance liquid chromatography (HPLC) was applied for the quantitative determination of the individual phenolic compounds. Sub-fraction 2 has the highest phytochemical contents as well as antioxidant activity. All tested phytochemicals had positively correlated to antioxidant activity. HPLC analysis showed that the phytochemicals in the fractionated extracts varied by the sugarcane cultivars. The main flavonoid substances found in the fractionated extracts were epicatechin, catechin, quercetin, resveratrol, myricetin and rutin while gallic acid was the main phenolic substance. The obtained information is useful for further studies and applications.

**Keywords: Partial purification, Fraction, Phytochemicals, Silica gel, Antioxidant activity.**

## INTRODUCTION

An economic crop in many countries worldwide including Thailand is sugarcane (*Saccharum officinarum* L.). It is known as a plant for sugar production. However, sugarcane is also used in folk medicine since it is composed of many types of phytochemicals [1] including phenolic compounds and flavonoids [2-6]. With previous work, various biological activities such as antioxidant, antiseptic, bactericide, cardiotonic, laxative properties of sugarcane extract were reported [7]. However, the phytochemicals found in sugarcane or its products were varied depending upon cultivars and geographic area [8,9].

All parts of sugarcane have also been reported for composing bioactive compounds and their bioactivity. The previous reports indicated that the total triterpenoid, phenolic and sterol varied by the parts and sugarcane cultivars. The phytochemicals showed a positive correlation with DPPH and FRAP antioxidant methods [3]. Bagasse is the residual or byproducts from the sugar production process. The bagasse is composed of hemi-cellulose, which is used for porous cellulose preparation [10]. The saccharides from bagasse exhibited antioxidant activity [11] and α-glucosidase inhibition activity [12]. The different solvent extracts of the sugarcane flower were investigated for their phytochemicals. The results found that the extracts were composed of various substances including alkaloids, tannins, anthraquinones, reducing sugar, saponins, flavonoids, polyphenols, steroids and terpenoids. Moreover, the finding substances exhibited lipid peroxidation [13]. The crude extract of molasses by ethanol (ME) and fractionated extract (MERBF) were investigated for antioxidant activity by various methods including ABTS, ORAC and CAA assays. The results indicated that the MERBF had higher antioxidant activity than the ME. In addition, the MERBF is composed of 13-types polyphenols which showed good activity for health supplement [14].

In Maha Sarakham, Thailand, many cultivars of sugarcane were planted for sugar production. We previously studied oxidative compounds in the Maha Sarakham [9]. Therefore, various cultivars of sugarcane in Maha Sarakham province were extracted and fractionated before investigation for their phyto-chemicals and antioxidant activity. The results affected

by cultivars and part of the sugarcane were revealed and discussed. The obtained results would be enhanced the information for sugarcane substances as well as their applications.

## EXPERIMENTAL

The different sugarcane cultivars were received and classified by the Agricultural Research and Development Center Mahasarakham, Maha Sarakham Province, Thailand. The samples were dried before grinding into small pieces for the further experimental process.

**Crude extract and fractionation:** The crude extracts of sugarcane were prepared by following the previous report [9] in triplicate for each sugarcane cultivar. The prepared crude extract was then fractionated throughout the silica gel column as described previously [15]. The sub-fractions were collected and identified the phytochemical were by absorbance measuring at 280 nm.

**Phytochemical determination:** The different phytochemicals; including total phenolic content (TPC) [16], total flavonoid content (TFC) [17], total saponin content (TSC) [18], total triterpenoid content (TTC) [19], total condensed tannins content (CDT) [20] were determined spectrophotometrically.

**Antioxidant potentials:** The antioxidant potentials of the fractionated extracts were evaluated with 2,2′-diphenyl-1-picrylhydrazyl (DPPH) [21], 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [16] and ferric-reducing/antioxidant power (FRAP) [22] protocols as described by previous reports.

**Phenolic compounds quantification:** The individual oxidative substances were analyzed by RP-HPLC analysis followed by reported method [17]. The external standards; gallic acid, catechin, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, myricetin, resveratrol and quercetin were used for identification.

**Statistical analysis:** The data were collected by triplicate measurements and expressed as mean ± standard deviation (SD) and significant differences with $p < 0.05$.

## RESULTS AND DISCUSSION

**Phenolic substances:** Plant are the important sources of phytochemicals that have been long used for traditional medicines [16]. The phenolic substances of the fractionated extracts have different types and contents which varied by the eluted fractions as shown in Table-1. The results found that all tested phytochemicals did not detect in SF1 (eluted by ethyl acetate) and SF5 (eluted by ethanol). The sub-fraction 2 (SF2), eluted by the mixture of ethyl acetate/ethanol at 25/75 (v/v) of all sugarcane cultivars composed similar TPC in the range of 1.15-1.23 mg GAE/g DW. The TFC found the top value in the SF2 than other fractions in all cultivars. Comparison between cultivars, KK-3 showed the highest TFC (3.50 mg CE/g DW). The TTC showed different patterns among the fractionated extracts. The highest TTC was found in KK-3 cultivar in SF2, but KK-80 and KK-07-037 found the highest in SF4. Total saponin content (TSC) found in KK-3 is higher than in other cultivars with in orders of SF2 > SF3 > SF4. The SF2 of Au-15, KK-3 and KK-80 composed the highest CDT, while KK-07-037 found the highest in SF4. Among cultivars, the KK-80 had the highest content (18.56 mg CE/g DW).

The plant's phytochemicals were also known for their biological activity and widely used as medicinal remedies [17,19]. Most of the phytochemicals are called secondary metabolites of plants which produced defence mechanism [12]. Flavonoids are found in high contents in plants and reported for various biological effects [23-26]. Plants including sugarcane composed of many types of phenolic compounds [3]. The secondary metabolites, especially phenolic acids and flavonoids responding for colour and taste. The saponins possess various activities for infection, which are already used as a key ingredients of medicine [27,28]. The tannins showed inhibitory effects on many types of enzymes such as an angiotensin-converting enzyme (ACE), trypsin and chymotrypsin [29], tyrosinase [30], α-amylase and α-glucosidase [31]. Tannins also helped to increase the number of high-density lipoproteins

TABLE-1
ACTIVE SUBSTANCES CONTENT (mg/g DW) IN THE SUB-FRACTIONS OF FOUR SUGARCANE CULTIVARS

| Cultivars | TFC | TPC | TTC | TSC | CDT |
|---|---|---|---|---|---|
| AU-15 | | | | | |
| SF2 | 1.46 ± 0.02$^{g}$ | 1.16 ± 0.02$^{d}$ | 10.85 ± 0.37$^{h}$ | 15.91 ± 0.42$^{ef}$ | 8.01 ± 0.30$^{g}$ |
| SF3 | 1.29 ± 0.02$^{g}$ | 0.76 ± 0.06$^{fg}$ | 11.55 ± 1.21$^{gh}$ | 10.83 ± 0.38$^{hi}$ | 5.16 ± 0.35$^{h}$ |
| SF4 | 1.03 ± 0.08$^{i}$ | 0.89 ± 0.04$^{ef}$ | 12.52 ± 1.05$^{gh}$ | 9.52 ± 0.65$^{i}$ | 6.26 ± 0.38$^{gh}$ |
| KK-3 | | | | | |
| SF2 | 3.50 ± 0.07$^{d}$ | 1.23 ± 0.05$^{d}$ | 59.19 ± 3.09$^{d}$ | 25.91 ± 0.38$^{c}$ | 13.45 ± 0.53$^{e}$ |
| SF3 | 1.15 ± 0.06$^{hi}$ | 0.85 ± 0.02$^{efg}$ | 23.96 ± 1.54 $^{efg}$ | 12.94 ± 0.34$^{fghi}$ | 8.08 ± 0.51$^{g}$ |
| SF4 | 1.39 ± 0.06$^{g}$ | 1.02 ± 0.01$^{de}$ | 27.33 ± 1.12$^{ef}$ | 19.33 ± 0.82$^{de}$ | 11.18 ± 1.11$^{f}$ |
| KK-80 | | | | | |
| SF2 | 2.47 ± 0.06$^{f}$ | 1.18 ± 0.05$^{d}$ | 21.55 ± 0.56$^{efgh}$ | 15.74 ± 1.64$^{ef}$ | 18.56 ± 0.61$^{d}$ |
| SF3 | 0.58 ± 0.03$^{j}$ | 0.65 ± 0.03$^{g}$ | 12.34 ± 0.36$^{gh}$ | 15.32 ± 0.77$^{efg}$ | 5.96 ± 0.31$^{h}$ |
| SF4 | 0.70 ± 013$^{j}$ | 0.69 ± 0.01$^{fg}$ | 17.63 ± 1.45e$^{fgh}$ | 10.69 ± 0.51$^{hi}$ | 6.61 ± 0.41$^{gh}$ |
| KK-07-037 | | | | | |
| SF2 | 3.27 ± 0.10$^{e}$ | 1.15 ± 0.06$^{d}$ | 15.39 ± 0.98$^{fgh}$ | 20.46 ± 0.99$^{d}$ | 7.98 ± 0.47$^{g}$ |
| SF3 | 0.77 ± 0.10$^{j}$ | 0.63 ± 0.01$^{g}$ | 9.04 ± 0.33$^{h}$ | 11.13 ± 0.42$^{ghi}$ | 3.10 ± 0.10$^{i}$ |
| SF4 | 0.76 ± 0.11$^{j}$ | 0.72 ± 0.02$^{fg}$ | 28.39 ± 0.45$^{e}$ | 14.83 ± 0.72$^{fgh}$ | 12.02 ± 1.16$^{ef}$ |

Results are done by triplicate measurements and expressed as mean ± SD. Significant differences at $p < 0.05$ showed by different letters in the same column represent. AU-15, Authong 15; KK-3, Khon Kaen 3; KK-80, Khon Kaen 80, KK-07-037, Khon Kaen 07-037; SF, sub-fraction.

and reduced low-density lipoprotein [32]. In addition, tannins can be inhibited the propagation of cancer cells HCT-15 [33]. Triterpenoids have been reported for their various biological activity [34]. In recent, triterpenoids were applied for medicinal remedies including diabetes, HIV and free radicals [9]. In this work, the fractionated extracts found phenolic acids, flavonoids, condensed tannins, saponins and triterpenoids. This indicated that this studied a good source of phytochemicals like other plants. Moreover, the triterpenoids, a first reported so far in the sugarcane by our research group was also found after fractionation by silica gel. The triterpenoid contents in sugarcane differed from apple, grape or olive [9]. It this not surprise according that they have many factors that affect the types and contents of phytochemicals [35,36]. These results confirmed that the sugarcane cultivars influenced the variable phytochemical contents. This was in agreement with the previous report [37].

**Antioxidant activity potentials:** The antioxidant activity competency of the fractionated extracts is shown in Table-2. With DPPH and ABTS scavenging methods, the sub-fraction SF2 of all cultivars had the lowest $IC_{50}$ value than other fractions. The lowest value by DPPH was found in the Au-15 (1.09 mg/mL), while ABTS found in KK-07-037 (7.78 mg/mL). The lowest $IC_{50}$ means higher competency of antioxidant activity. In general, the ABTS assay exhibited a lower $IC_{50}$ value than the DPPH assay. FRAP assay indicated that the sub-fraction SF2 revealed the highest potential than other fractions in all the cultivars. The most competency for ferric ion reducing found in the SF2 of KK-3 (341 μM $FeSO_4$/g DW), then KK-07-037 (202 μM $FeSO_4$/g DW), KK-80 (194 μM $FeSO_4$/g DW) and Au-15 (163 μM $FeSO_4$/g DW), respectively.

Previous works indicated that the antioxidant activity of the phytochemicals was investigated by different methods. Since no one assay that could be known for all active mechanisms as the structures of phytochemicals are very complex [38,39]. The competency of antioxidant activity was concerned by the chemical structure of phytochemicals as well as their functional group components [40,41]. $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals scavenging assays were the most popularly done for phytochemicals antioxidant activity test [38,42,43]. The results showed the variable value of antioxidant activity by sub-fractions and sugarcane cultivars. However, the obtained activity was higher competency than the previous report [3]. The variable activity was directly concerned with the type and content of substances. FRAP was a reducing power method for the investigation of antioxidant activity. This power was usually tested as a scavenging activity. The fractionated extracts of sugarcane showed high value by FRAP assay, especially SF2. This means the fractionated extracts could be protected the ferric ion to promote the onset of free radicals by changing ferric to ferrous ion. The obtained results were in agreement with the previous report which suggested that *ortho*-dihydroxyl polyphenols could be reacted to $Fe^{2+}$ or metal *via* coordination mechanism [44].

**Individual phenolic compounds:** Most oxidative compounds were found in the sub-fraction SF2. Therefore, the SF2 was then identified for the individual phenolic compounds using HPLC. The 10 phenolic standards were used as external standard and the results are shown in Table-3. The AU-15 found the highest content of epicatechin (4.18 mg/g), quercetin (1.77 mg/g), ferulic acid (1.47 mg/g), resveratrol (0.87 mg/g) for phenolic acids while gallic acid (0.74 mg/g) and catechin (0.41 mg/g) were the major flavonoid, respectively. KK-3 cultivar found quercetin (2.58 mg/g), in the highest content and then resveratrol (2.52 mg/g), epicatechin and ferulic acid (1.8 mg/g), *p*-coumaric acid (1.76 mg/g), rutin (1.02 mg/g), gallic acid (0.74 mg/g), myricetin (0.50 mg/g) and catechin (0.37 mg/g), respectively. The phenolic compounds in the KK-80 cultivar indicated that gallic acid (7.65 mg/g) was the main type, followed by catechin (4.88 mg/g), ferulic acid (2.36 mg/g), epicatechin (0.75 mg/g), resveratrol (0.71 mg/g) and quercetin (0.67 mg/g), respectively. KK-07-037 cultivar found the epicatechin (2.94 mg/g) as the main type and then ferulic acid (2.56 mg/g), quercetin (1.44 mg/g), gallic acid (1.12 mg/g), resveratrol (0.64 mg/g), catechin (0.37 mg/g) and myricetin (0.34 mg/g), respectively. HPLC results indicated that the main phenolic acids in the sugarcane fractionated extracts were epicatechin, catechin, quercetin, resveratrol, myricetin and rutin were the main flavonoids while gallic acid, ferulic acid and *p*-coumaric acid. Flavonoids have been reported as the highest contents [45]. The catechin and epicatechin have been reported as the main flavonoids in plants [46]. These substances were applied for cancer therapy [47]. Myricetin, a flavonol was found generally in low content [48]. Resveratrol

TABLE-2
ANTIOXIDANT ACTIVITY IN THE FRACTIONATED EXTRACTS OF FOUR SUGARCANE CULTIVARS

| Cultivars | Methods | | | | | |
|---|---|---|---|---|---|---|
| | DPPH ($IC_{50}$ mg/mL) | ABTS ($IC_{50}$ mg/mL) | FRAP (μM $FeSO_4$/g DW) | DPPH ($IC_{50}$ mg/mL) | ABTS ($IC_{50}$ mg/mL) | FRAP (μM $FeSO_4$/g DW) |
| | Au-15 | | | KK-3 | | |
| SF2 | $1.09 \pm 0.01^{k}$ | $15.27 \pm 0.50^{d}$ | $162.80 \pm 3.03^{d}$ | $2.95 \pm 0.13^{i}$ | $14.04 \pm 0.80^{e}$ | $341.55 \pm 7.78^{a}$ |
| SF3 | $5.75 \pm 0.14^{g}$ | $32.72 \pm 0.94^{s}$ | $122.97 \pm 5.17^{fg}$ | $6.57 \pm 0.14^{f}$ | $15.91 \pm 0.39^{d}$ | $112.06 \pm 1.06^{hi}$ |
| SF4 | $3.80 \pm 0.02^{h}$ | $29.33 \pm 0.58^{b}$ | $162.28 \pm 6.13^{d}$ | $12.43 \pm 0.28^{d}$ | $21.82 \pm 1.01^{c}$ | $158.41 \pm 1.56^{d}$ |
| | KK-80 | | | KK-07-037 | | |
| SF2 | $2.61 \pm 0.08^{ij}$ | $11.73 \pm 0.77^{f}$ | $194.70 \pm 1.17^{c}$ | $2.24 \pm 0.13^{j}$ | $7.78 \pm 0.28^{h}$ | $202.43 \pm 5.37^{b}$ |
| SF3 | $9.30 \pm 0.12^{e}$ | $13.41 \pm 0.54^{e}$ | $144.12 \pm 4.54^{e}$ | $15.59 \pm 0.24^{b}$ | $28.66 \pm 0.64^{b}$ | $113.95 \pm 3.10^{hi}$ |
| SF4 | $26.41 \pm 0.78^{a}$ | $15.94 \pm 0.36^{d}$ | $114.01 \pm 1.35^{hi}$ | $13.09 \pm 0.24^{c}$ | $31.92 \pm 0.67^{a}$ | $119.52 \pm 1.57^{gh}$ |

Results are done by triplicate measurements and expressed as mean ± SD. Significant differences at $p < 0.05$ showed by different letters in the same column represent.

TABLE-3
PHENOLIC COMPOUNDS IN THE SUGARCANE FRACTIONATED EXTRACTS EXPRESSED AS mg/g DW

| Cultivars | Gallic acid | Catechin | Caffeic acid | Epicatechin | *p*-Coumaric acid |
|---|---|---|---|---|---|
| Au-15 | 1.30 ± 0.12$^{cd}$ | 7.54 ± 1.15$^{d}$ | 0.41 ± 0.25$^{a}$ | 1.93 ± 0.16$^{c}$ | 0.13 ± 0.02$^{bc}$ |
| SF2 | 0.74 ± 0.09$^{e}$ | 0.41 ± 0.05$^{f}$ | 0.12 ± 0.01$^{d}$ | 4.18 ± 0.43$^{a}$ | 0.04 ± 0.00$^{c}$ |
| KK-3 | 1.27 ± 0.07$^{cde}$ | 43.78 ± 3.46$^{a}$ | 0.34 ± 0.00$^{c}$ | 1.22 ± 0.07$^{de}$ | 0.21 ± 0.01$^{b}$ |
| SF2 | 0.88 ± 0.09$^{de}$ | 0.37 ± 0.09$^{f}$ | 0.12 ± 0.02$^{d}$ | 1.88 ± 0.72$^{c}$ | 1.76 ± 0.23$^{a}$ |
| KK-80 | 3.23 ± 0.41$^{b}$ | 23.60 ± 2.14$^{b}$ | 0.37 ± 0.00$^{b}$ | 0.82 ± 0.14$^{e}$ | 0.09 ± 0.00$^{bc}$ |
| SF2 | 7.65 ± 0.68$^{a}$ | 4.88 ± 0.68$^{e}$ | 0.08 ± 0.00$^{e}$ | 0.75 ± 0.11$^{e}$ | 0.03 ± 0.00$^{c}$ |
| KK-07-037 | 1.75 ± 0.07$^{c}$ | 10.82 ± 0.45$^{c}$ | 0.37 ± 0.02$^{b}$ | 1.48 ± 0.06$^{cd}$ | 0.13 ± 0.01$^{bc}$ |
| SF2 | 1.12 ± 0.04$^{de}$ | 0.37 ± 0.03$^{f}$ | 0.10 ± 0.01$^{de}$ | 2.94 ± 0.20$^{b}$ | 0.04 ± 0.00$^{c}$ |
| Cultivars | Ferulic acid | Rutin | Myricetin | Resveratrol | Quercetin |
| Au-15 | 3.80 ± 0.48$^{b}$ | 0.24 ± 0.01$^{c}$ | 0.31 ± 0.09$^{c}$ | 6.74 ± 1.36$^{b}$ | 11.40 ± 1.25$^{b}$ |
| SF2 | 1.47 ± 0.30$^{d}$ | 0.10 ± 0.02$^{d}$ | 0.12 ± 0.01$^{e}$ | 0.87 ± 0.09$^{d}$ | 1.77 ± 0.13$^{d}$ |
| KK-3 | 10.61 ± 0.03$^{a}$ | 0.49 ± 0.07$^{b}$ | 1.11 ± 0.08$^{a}$ | 8.39 ± 1.22$^{a}$ | 30.51 ± 2.62$^{a}$ |
| SF2 | 1.80 ± 0.10$^{d}$ | 1.02 ± 0.13$^{a}$ | 0.50 ± 0.16$^{b}$ | 2.52 ± 0.21$^{c}$ | 2.58 ± 0.20$^{d}$ |
| KK-80 | 3.40 ± 0.32$^{b}$ | 0.10 ± 0.00$^{d}$ | 0.04 ± 0.00$^{e}$ | 6.59 ± 0.68$^{b}$ | 5.02 ± 0.43$^{c}$ |
| SF2 | 2.36 ± 0.07$^{c}$ | 0.08 ± 0.01$^{d}$ | 0.27 ± 0.04$^{cd}$ | 0.71 ± 0.03$^{d}$ | 0.67 ± 0.08$^{d}$ |
| KK-07-037 | 3.40 ± 0.20$^{b}$ | 0.11 ± 0.03$^{d}$ | 0.14 ± 0.04$^{de}$ | 7.46 ± 0.97$^{ab}$ | 6.29 ± 0.54$^{c}$ |
| SF2 | 2.56 ± 0.14$^{c}$ | 0.09 ± 0.02$^{d}$ | 0.34 ± 0.06$^{c}$ | 0.64 ± 0.05$^{d}$ | 1.44 ± 0.08$^{d}$ |

Results are done by triplicate measurements and expressed as mean ± SD. Significant differences at $p < 0.05$ showed by different letters in the same column represent. ND = not detected.

was a kind of flavonoid found only in fruit peels [49,50]. However, this flavonoid was not detected in the sub-fraction of the fractionated extracts. This revealed that the phytochemicals varied by different factors including cultivar, growth stage, colour, part, genetic, climate and geographic area [9,51,52].

## Conclusion

These fractionated extracts of sugarcane planted in Maha Sarakham province of Thailand are composed of various natural oxidative compounds, especially total saponin. The phytochemicals and antioxidant activity found variable values depending on the sugarcane cultivars. However, the sub fraction SF2 showed the highest phytochemical contents as well as the antioxidant activity. This gallic acid, ferulic acid and *p*-coumaric acid are found in the SF2 as main phenolic acids while flavonoids were epicatechin, quercetin, resveratrol and catechin, respectively. The cultivars of sugarcane planted in Maha Sarakham province are good sources of oxidative phytochemicals that would be applied for health and cosmetics, except sugar production.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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