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Research Article
Antipathogenic Properties and Antioxidant Activity of Black Sugarcane (*Saccharum officinarum* L.): Biological Activities of Black Sugarcane Cultivars

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Abstract
Background and Objective: Black sugarcane (*Saccharum officinarum* L.) is characterized by its distinctive dark-colored stems or joints containing of bioactive compounds. These compounds are associated with potential antioxidant and antibacterial activities, indicating possible health-promoting properties. This research aimed to investigate the phytochemical contents in the two cultivars of black sugarcane and to screen for their antioxidant, antibacterial and tyrosinase inhibition activities. **Materials and Methods:** Dried *S. officinarum* was extracted with methanol: HCl. The crude extract was used for phytochemical contents, antioxidant activity, phenolic compound constituents, tyrosinase inhibition and antibacterial activity assay. This experimental design followed by descriptive analysis and were presented as Mean$\pm$Standard Deviation (SD). **Results:** The total phenolic compounds found in the extracts were arranged of 12.36-23.37 mg CE/g which is found in small joints higher than in big joint cultivars. The big joint found the highest activity for DPPH$^{\bullet}$ radical in root extract (0.52 mg/mL). The root extracts of the small joint showed the highest ABTS$^{\bullet +}$ radical scavenging activity at 0.33 mg/mL. Catechin was the main compound in the black sugarcane which was higher in the big joint than the small joint variety. The extracts from both black sugarcane varieties showed tyrosinase inhibition activity. The lowest MIC and MBC values at 0.39 and 0.05 mg/mL were obtained from the node and rind of the small joint against *Klebsiella pneumoniae*. **Conclusion:** This result indicated that the black sugarcane was composed of phytochemicals that expressed high antioxidant, tyrosinase inhibition and antibacterial activities. It might further use black sugarcane extracts for health, beauty and pharmaceutical applications.

Key words: *Saccharum officinarum* L., antibacterial activity, antioxidation, *Klebsiella pneumoniae*, tyrosinase inhibition activities

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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

Sugarcane (*Saccharum officinarum* L.), a monocot plant belonging to the Poaceae family, is one of the attractive crops since it is an essential source of sugar and ethanol production[1-3]. Thailand is the fourth biggest producer in the world[4]. On the other hand, various studies identified a lot of active substances in sugarcane and its products[5-10]. These active compounds have been widely used in various fields such as skin care ingredients[2], modulators for inflammation and neurological disorders[5], cosmetics and medical products[11], food and pharmaceuticals and regulate carbohydrate metabolism[12,13].

Previous studies show that different active compounds could be detected in all parts of sugarcane, including its juice[14-16]. The Northeastern Region of Thailand has many sugarcane cultivars both cultivated and wild spp. The cultivated sugarcane is planted as a material source for the sugar industry. The wild type of sugarcane commonly had colors in its stem; red and black. It is not a popular cultivation for juice or other purposes. This is according to the small amount of juice as well as its taste. This wild sugarcane is known as a medicinal plant and used as ingredients in folk medicine. Black sugarcane, a wild type of sugarcane which grows by themselves in the land spaces, is an attractive study. It has been used as a medicinal plant for folk medicine for treatment of various symptoms by local people. This purpose was the same as any other country in the world which believed in folk medicine[16,17]. However, scientific study on black sugarcane phytochemicals and their biological activities has a few reported. The objectives of this work were to investigate the phytochemical contents in the two cultivars of black sugarcane and screen for their antioxidant, antibacterial and tyrosinase inhibition activities. The effects of cultivars and parts of the sugarcane were compared and discussed.

MATERIALS AND METHODS

Study area: This research was performed from October 2021 to August 2023 in The Center of Excellence Innovation in Chemistry (PERCH-CIC) and Biodegradable Polymers Research Unit, Department of Chemistry, Faculty of Science, Mahasarakham University and the Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Black sugarcane collection and preparation: Two cultivars of black sugarcane were collected from a village directly in Maha Sarakham Province, Thailand. Both sugarcane samples were separated (tip, node, rind and root) and dried in air and cut into small pieces for extraction. The dried sugarcane samples were mixed with methanol: HCl (99:1 v/v) at a 1:20 (w/v) ratio to prepare crude extract by maceration for 24 hrs. The triplicate times were performed for each part of the samples. The obtained crude extract was dissolved in methanol before analysis of phytochemical contents, antioxidant activity, phenolic compounds constituents and tyrosinase inhibition assay. The crude extract was dissolved in Dimethyl Sulfoxide (DMSO, Sigma) to the final concentration at 500 mg/mL before antibacterial activity determination.

Determination of total phytochemical contents: The spectrophotometric method was used for the determination of different active substances; total flavonoid content (TFC) by using catechin as standard[18], total phenolic content (TPC) by using gallic acid as standard[19], total triterpenoid content (TTC) by using ursolic acid as standard[20], total saponin content (TSaC) by using aescin as standard[21] and total condensed-tannins content (CDT) by using catechin as standard[22].

Evaluation of antioxidant activity: Different mechanisms were performed to evaluate the antioxidant profiles of the extracts. Free radical scavenging activity on 2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)[23] was selected for this activity. In addition, metal-reducing power activity by ferric reducing/antioxidant power (FRAP)[24] was also chosen.

Identification of phenolic compounds constituents: The RP-HPLC analysis was performed for the identification of substance constituents in the extracts. The condition process and solvent eluted were conducted followed by the previous report by Sufian *et al.*[18]. The ten external standards were used for identification.

Tyrosinase inhibition assay: The enzymatic activity was measured by adaptation of the method described by Hawary *et al.*[24]. A 2 mL solution composed of 700 μL tyrosinase substrate (DOPA) in phosphate buffer solution, 700 μL tyrosinase enzyme in phosphate buffer and 600 μL extracts were prepared. The mixture was incubated at 30°C for 30 min. The reaction was measured at 475 nm. All tests were done in triplicate. Kojic acid was used as positive control and phosphate buffer was used as negative control. This activity was calculated as:

$$AI\ (\%) = \frac{C - S}{S} \times 100$$

Where:

C = Negative control absorbance
S = Sample or positive control absorbance[25]

The activity was expressed as the concentration of a sample that produces 50% inhibition of enzymatic activity (IC_{50}) which is calculated by plotting AI (%) against concentration mg/mL.

Pathogenic bacteria preparation: A single colony of each bacteria including *Staphylococcus aureus* TISTR 1466, *Bacillus cereus* TISTR 2737, *Staphylococcus aureus* PK and *Klebsiella pneumoniae* was transferred to 5 mL culture broth medium and incubated at 37°C with shaking for 24 hrs. The bacterial cell concentration was adjusted at OD_{600} to 0.1 before use.

Disc diffusion method: One hundred of each tested bacterium (OD_{600} to 0.1) were spread onto nutrient agar (NA) and a sterile paper dish with a 0.6 mm diameter was placed on NA. Ten microliters of ethanolic *Saccharum officinarum* L. extract were dropped onto the paper disc (triplicate). The plate was incubated at 37°C for 24 hrs. The inhibition zone around the disc was measured and recorded.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination: The MIC and MBC values of *S. officinarum* L. extract against pathogenic bacteria were determined. One hundred microliters of NB were added to each well and the *S. officinarum* L. extract was twofold serially diluted. One hundred microliters of each bacterium were added to each well. The plate was incubated at 37°C for 24 hrs before MIC and MBC values were measured by colorimetric assay. The iodonitrotetrazolium (INT) solution was added to each well and incubated for 30 min. The wells without bacteria are yellow in color and the wells with bacterial growth turned pink in color[26-28].

Statistical analysis: All tests were conducted in triplicate. The results were presented as Mean±Standard Deviation (SD). The statistical significance test differences with $p<0.05$ were assigned.

RESULTS AND DISCUSSION

Phenolic compounds in extracts: Sugarcane is composed generally of various types of secondary metabolites which can be used for traditional medicine with a long history[2,10,11,14]. Table 1 shows the total phenolic substances in each part of two black sugarcane cultivars. The results found that the tested substances were variable profiles depending on parts and cultivars. The TFC found in the extracts were arranged at 12.36-23.37 mg CE/g which is found in small joints higher than big joint cultivars, except in shoot tip extract. Considering each part of the black sugarcane, the root extract of small joints has the highest TFC (23.37 mg CE/g), meanwhile, the shoot tip extract has the lowest TFC (12.36 mg CE/g). In contrast, the tip extract of the big joint has the highest TFC (21.79 mg CE/g). The TPC in the black sugarcane is found in lower content than TFC in the range of 6.61-14.50 mg GAE/g. The TPC in small joints is found slightly in higher content than in big joints. The rind extract of the small joint cultivar was found the highest TPC (14.50 mg GAE/g) whereas the big joint was found in the shoot tip extract (12.51 mg GAE/g). The TTC is also found with low content and variable patterns among the parts of extracts (0.21-0.83 mg UR/g). The highest TTC was found in the root of the small joint cultivar (0.83 mg UR/g), but other parts found lower TTC than in big joints. The node of the big joint contained the highest TTC (0.74 mg UR/g) among other parts of this cultivar. Total saponin content (TSaC) found in the range of 10-24 mg AES/g. The root (24.52 mg AES/g) and rind (18.02 mg AES/g) of small joint extracts contained higher TSaC than the big joint extracts, while node and tip extracts were in lower content. The CDT in the extracts was arranged in 6.66-18.90 mg CE/g. In general, all parts of the small joint cultivar are composed of higher CDT than big joints, except for the shoot tip. The root of the small joint composed the highest CDT (18.90 mg CE/g), as well as the big joint (11.81 mg CE/g DW).

Humans have known for a long history to use medicinal plants in their life[18,20]. The different plant phytochemicals involved in defense and reproduction propose[29]. There are many reports of their activities[29-32]. The selected black sugarcane extracts are composed of all the mentioned compounds with high content. In addition, the triterpenoids were also detected even in low content if compared to other plants[33]. This revealed that the phytochemical contents were varied by parts as well as plant cultivars[2,14,34].

Antioxidant determination: Table 2 shows the antioxidant activity results of the black sugarcane extracts comparing

Table 1: Total phenolic constituents (mg/g DW) in black sugarcane extracts

Substances	TFC	TPC	TTC	TSaC	CDT
Small joint					
Tip	12.36±0.72[d]	9.87±0.09[c]	0.21±0.01[f]	10.18±0.09[e]	6.66±0.12[e]
Node	18.05±1.74[b]	8.92±0.86[c]	0.34±0.02[e]	10.06±0.30[e]	9.46±0.32[d]
Rind	18.96±1.86[b]	14.50±0.18[a]	0.46±0.02[d]	18.02±0.58[b]	14.35±0.75[b]
Root	23.37±3.24[a]	12.90±0.86[b]	0.83±0.07[a]	24.52±0.67[a]	18.90±2.60[a]
Big joint					
Tip	21.79±1.83[a]	12.51±1.21[b]	0.56±0.02[c]	11.64±0.7[d]	8.72±0.63[d]
Node	14.77±1.15[c]	8.18±0.39[c]	0.74±0.01[b]	12.56±0.24[c]	8.47±3.24[d]
Rind	12.98±0.28[d]	7.35±0.49[d]	0.56±0.04[c]	9.91±0.55[e]	7.85±0.52[d]
Root	12.89±0.14[d]	6.61±0.15[d]	0.61±0.01[c]	17.86±0.31[b]	11.81±0.08[c]

Results are expressed as Mean±SD from triplicate values and statistical significance differences at $p<0.05$ showed with different letters in the same column represent

Table 2: Antioxidant efficacy in black sugarcane extracts

	Methods		
Cultivars	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	FRAP (µM $FeSO_4$/g DW)
Small joint			
Tip	4.19±0.01[a]	4.22±0.03[a]	349.31±24.01[e]
Node	1.77±0.10[b]	1.29±0.03[b]	370.21±20.94[e]
Rind	0.56±0.01[f]	0.77±0.01[d]	712.09±10.44[b]
Root	0.58±0.01[f]	0.33±0.03[h]	634.42±12.53[c]
Big joint			
Tip	0.89±0.02[c]	0.93±0.01[c]	803.28±9.25[a]
Node	0.82±0.02[d]	0.68±0.01[e]	551.23±25.50[d]
Rind	0.73±0.02[e]	0.46±0.02[f]	638.77±27.36[c]
Root	0.52±0.02[f]	0.41±0.01[g]	786.10±19.72[a]

Results are expressed as Mean±SD from triplicate values and statistical significance differences at $p<0.05$ showed with different letters in the same column represent

small and big joint cultivars. With $DPPH^{\bullet}$ testing, the small joint extracts had lower IC_{50} values than the big joint, except for the rind extract. This revealed that the small joint had higher antioxidant potential than the big joint. Considering each part of the sugarcane, the root and rind extracts had the highest $DPPH^{\bullet}$ scavenging activity for the small joint (0.56, 0.58 mg/mL) while the big joint found the highest activity for $DPPH^{\bullet}$ radical in root extract (0.52 mg/mL). Testing for $ABTS^{\bullet+}$ radical scavenging activity indicated that almost all parts of big joint extracts had higher potential activity than small joints, except in root extract. The root extracts of both small joint and big joint showed the highest $ABTS^{\bullet+}$ radical scavenging activity as 0.33 and 0.41 mg/mL, respectively. Moreover, the extracts from all parts of big joint showed generally free radical scavenging for $ABTS^{\bullet+}$ in lower IC_{50} values than $DPPH^{\bullet}$. This mean that the small joint extracts had higher potency for $ABTS^{\bullet+}$ than $DPPH^{\bullet}$. In case of FRAP assay, the extracts from all parts of big joint black sugarcane showed higher reducing power than small joint extracts. The most competency was found in the tip extract of big joint (803.28 µM $FeSO_4$/g), then root (786.10 µM $FeSO_4$/g), rind (638.77 µM $FeSO_4$/g) and node (551.23 µM $FeSO_4$/g DW), respectively. The extracts from small joint also contained high reducing activity with the highest in rind (712.09 µM $FeSO_4$/g), then root (634.42 µM $FeSO_4$/g), node (370.21 µM $FeSO_4$/g) and tip (349.31 µM $FeSO_4$/g DW), respectively.

It is known that phytochemical's structures were complexity[35,36]. Therefore, antioxidant tests should be carried out by various methods. Most reports indicated that the functional components of phytochemicals are directly related to the competency of antioxidant activity[37,38]. Free radicals scavenging[35,38-40] and FRAP[41] assays were the most popular methods for antioxidant determination. From the results, the variable potency of antioxidants is depending on sugarcane cultivars and parts. However, it can suggest that black sugarcane is composed of higher antioxidant competency than the previous reports on sugarcane and they're by-products[14,15,42]. The high antioxidant competency might be caused by those flavonoids in the extracts, especially big joint cultivars. This is due to the ortho-dihydroxy polyphenols substances like flavonoids or saponins in the extracts[15,41,42].

Quantitative analysis of phenolic compounds: The retention time of 10 reference standards was used for quantitative analysis of phenolic compounds using HPLC as shown in Table 3. In small joint cultivars, the main phenolic compounds were catechin, quercetin, ferulic acid, epicatechin, resveratrol and gallic acid which were variants of contents depending on the parts. The catechin was found to have the highest content in the shoot tip (50.52 mg/g) followed by node (45.60), root (40.48) and rind (38.55). Quercetin found the highest content in root (25.85 mg/g), followed by ride (13.30), node (6.90) and

Table 3: Abundance of phenolic constituents (mg/g DW) in black sugarcane extracts

Cultivars	Gallic acid	Catechin	Caffeic acid	Epicatechin	*p*-Coumaric acid
Small joint					
Tip	1.08±0.03^{c}	50.52±0.69^{b}	0.50±0.01^{cd}	2.21±0.15^{c}	0.13±0.00^{f}
Node	1.55±0.35^{c}	45.60±3.10^{bc}	1.16±0.17^{b}	5.07±0.76^{a}	0.77±0.02^{df}
Rind	2.61±0.17^{b}	38.55±0.50^{d}	0.95±0.05^{bc}	5.56±0.04^{a}	0.26±0.04^{ef}
Root	3.22±0.12^{a}	40.48±0.71^{cd}	1.01±0.02^{bc}	0.60±0.01^{d}	1.02±0.00^{cd}
Big joint					
Tip	1.55±0.02^{c}	83.39±1.83^{a}	1.83±0.15^{a}	2.89±0.06^{bc}	1.63±0.13^{b}
Node	1.51±0.60^{c}	87.01±3.91^{a}	0.34±0.02^{d}	2.96±0.24^{bc}	1.50±0.12^{bc}
Rind	1.36±0.09^{c}	80.43±4.29^{a}	0.93±0.52^{bc}	3.72±0.47^{b}	5.17±0.64^{a}
Root	1.75±0.01^{c}	80.85±3.34^{a}	0.35±0.00^{d}	0.90±0.22^{d}	0.56±0.13^{def}
Cultivars	Ferulic acid	Rutin	Myricetin	Resveratrol	Quercetin
Small joint					
Tip	2.73±0.12^{c}	0.29±0.01^{d}	0.45±0.04^{b}	2.19±0.24^{d}	6.29±0.61^{d}
Node	2.28±0.02^{c}	0.11±0.01^{e}	0.17±0.02^{b}	2.38±0.05^{d}	6.90±0.18^{d}
Rind	4.13±0.70^{b}	1.05±0.07^{b}	0.69±0.06^{b}	5.26±0.23^{a}	13.30±0.22^{c}
Root	4.59±0.02^{b}	1.35±0.03^{a}	1.82±0.03^{a}	4.16±0.33^{b}	25.87±0.59^{b}
Big joint					
Tip	7.81±0.97^{a}	0.48±0.05^{c}	0.59±0.04^{b}	4.93±0.12^{a}	39.57±2.28^{a}
Node	7.95±0.62^{a}	0.48±0.05^{c}	0.33±0.06^{b}	5.21±0.27^{a}	39.29±1.03^{a}
Rind	7.89±0.74^{a}	0.35±0.01^{d}	0.89±0.77^{b}	4.91±0.27^{a}	36.88±4.28^{a}
Root	3.42±0.29^{bc}	0.26±0.05^{d}	0.48±0.03^{b}	3.42±0.24^{c}	14.01±0.17^{c}

Results are expressed as Mean±SD from triplicate values and statistical significance differences at $p<0.05$ showed with different letters in the same column represent

shoot tip (6.29). Epicatechin was found the highest content in the rind (5.56 mg/g) followed by node (5.07), tip (2.21) and root (0.60). Resveratrol found the highest content in rind (5.26 mg/g) followed by root (4.16), node (2.28) and tip (2.19). Ferulic acid was a phenolic acid that contained the highest content in the root (4.16 mg/g) followed by rind (4.13), tip (2.73) and node (2.28). Gallic acid found the highest content in root (3.22 mg/g) followed by rind (2.61), node (1.55) and tip (1.08). In addition, root extract was also composed of high contents of other flavonoids; rutin, myricetin and phenolic acid; p-coumaric acid and caffeic acid.

In the big joint cultivar, the main phenolic compounds were also catechin, quercetin, ferulic acid, epicatechin, resveratrol and gallic acid which were variants of contents depending on the parts. Moreover, these substances contain higher content than in small joint cultivars, especially flavonoids. The catechin was found to have the highest content in nodes (87.01 mg/g) followed by tip (83.39), root (80.85) and rind (80.43), respectively. Quercetin was found the highest content in the shoot tip (39.57 mg/g), followed by node (39.29), rind (36.88) and root (14.01). Epicatechin found the highest content in rind (3.72 mg/g) followed by node (2.96), tip (2.89) and root (0.90). Resveratrol was found the highest content in nodes (5.21 mg/g) followed by tip (4.93), ride (4.91) and root (3.42). On the other hand, phenolic acids were also found in lower content, except ferulic acid. The ferulic acid was found the highest content in nodes (7.95 mg/g) followed by rind (7.89), tip (7.81) and root (3.42). Gallic acid was found the highest content in the root (1.75 mg/g) followed by tip (1.55), node (1.51) and rind (1.36). Moreover, all big joint extracts contained high contents of phenolic acids, especially p-coumaric acid and caffeic acid.

The sugarcane composed of different constituents with high contents[14], especially flavonoids. The obtained result was in agreement with previous reports suggesting that mono-flavonoids like catechin and epicatechin are the main plant substances[9-11]. Other flavonoids: Myricetin, resveratrol and rutin which are generally found in small continents[43] were also found in all parts of black sugarcane. Interestingly, p-coumaric acid and caffeic acid were also detected in the black sugarcane which agreed with previous work by researchers[10,11]. The type and level of tested phenolic compounds vary according to cultivars and parts such as extraction conditions and its byproducts[2,11].

Tyrosinase inhibition activity: Table 4 shows tyrosinase inhibition activity in each part of two black sugarcane cultivars. The tyrosinase inhibition activity of extracts was lower than standard kojic acid (0.0943 mg/mL). However, the results indicated high activity. Among the cultivars, almost of small joint extracts have higher tyrosinase inhibition activity than the extracts of big joints, except in tip extract. In comparison between parts of sugarcane, the root of the big joint has the highest activity, then the root and tip of the small joint. The node and rind of big joint extracts have a similar potent activity to the node extract of small joints. The obtained results were in lower tyrosinase inhibition activity of the rind extract of *Citrus mitis* Blanco (IC_{50} = 0.87 mg/mL)[44]

Table 4: Tyrosinase inhibition activity in black sugarcane extracts

Cultivars	IC_{50} (mg/mL)
Small joint	
Tip	1.84±0.05[b]
Node	1.96±0.09[c]
Rind	2.13±0.06[d]
Root	1.82±0.08[b]
Big joint	
Tip	2.49±0.09[e]
Node	1.99±0.02[c]
Rind	1.92±0.02[c]
Root	1.43±0.5[a]

Results are expressed as Mean±SD from triplicate values and significant differences at p<0.05 showed by different letters in the same column represent

Table 5: Diameter of inhibition zones (mm) of *Saccharum officinarum* L. extracts against pathogenic bacteria

	Inhibition zones (mm)			
Saccharum officinarum L.	*Staphylococcus aureus* TISTR 1466	*Bacillus cereus* TISTR 2737	*Staphylococcus aureus* PK	*Klebsiella pneumoniae*
Small joint				
Shoot tip	16	15	15	12
Node	15	13	14	13
Rind	13	11	13	11
Root	12	7	10	11
Big joint				
Shoot tip	-	7	7	13
Node	-	12	7	13
Rond	-	7	7	14
Root	11	7	10	13
DMSO	-	-	-	-

-: No antibacterial activity

Table 6: MIC and MBC value of *Saccharum officinarum* L. extracts against tested pathogenic bacteria

	Staphylococcus aureus TISTR 1466		*Bacillus cereus* TISTR 2737		*Staphylococcus aureus* PK		*Klebsiella pneumoniae*	
Saccharum officinarum L.	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Small joint								
Shoot tip	0.39	0.20	0.78	0.39	0.78	0.39	0.78	0.39
Node	0.39	0.10	0.78	0.39	0.78	0.39	0.39	0.05
Rind	0.39	0.20	0.39	0.20	0.78	0.39	0.39	0.05
Root	3.13	1.56	1.56	0.78	3.13	1.56	1.56	0.39
Big joint								
Shoot tip	-	-	1.56	0.78	3.13	1.56	1.56	0.78
Node	-	-	3.13	0.20	3.13	1.56	1.56	0.39
Rond	-	-	3.13	0.20	3.13	1.56	6.25	1.56
Root	3.13	1.56	1.56	0.78	3.13	1.56	1.56	0.78
DMSO	-	-	-	-	-	-	-	-

and seed of Hawthorn berry (IC_{50} = 0.87 μg/mL)[45]. It might be thought that black sugarcane extracts should be used as an active component in cosmetics for skin care and melasma protection[2].

Antibacterial activity: The highest inhibition zone diameter at 16 mm was presented in the small joint shoot tip extract against *S. aureus* TISTR 1466 followed by *B. cereus* TISTR 2737 and *S. aureus* PK with 15 mm (Table 5). The two black sugarcane cultivars' roots are the most suitable for antibacterial substance extraction because all root extracts can inhibit the growth of 4 tested bacteria. The *K. pneumoniae* was the most sensitive bacteria with all parts of small joint and big joint extracts. This result according to Zhao *et al.*[46], that reported the ethanolic sugarcane bagasse extracts showed the inhibition zone diameter at 19.4±0.15 mm against *S. aureus* ATCC 6538. They also reported that ethanolic sugarcane bagasse extracts can inhibit the growth of *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 8089 and *Salmonella typhimurium* ATCC 14028. Williams *et al.*[47] reported that methanol extract of *S. officinarum* stem pulp can inhibit the growth of

S. aureus ranging from 8.67-24.00 mm. The ethanolic small joint shoot tip and nod extracts from this work presented a higher inhibition zone compared with what Amalia *et al.*[48] (13.8 mm) and Uchenna *et al.*[49] reported.

The lowest MIC value at 0.39 mg/mL was presented in the small joint against *S. aureus* TISTR 1466 (shoot tip, node and rind), *B. cereus* TISTR 2737 (rind) and *K. pneumoniae* (node and rind) (Table 6). The lowest MBC at 0.05 mg/mL was shown in the node and rind of the small joint against *K. pneumoniae* (Table 6). The MBC against *S. aureus* TISTR 1466 was lower than reported by Zhao *et al.*[46].

CONCLUSION

Black sugarcane contained various oxidative substances which expressed high antioxidant capacities. The results suggested that cultivars and their parts of sugarcane were directly influenced by phytochemicals and antioxidant activity. However, all parts of the black sugarcane were a good source of phytochemicals. Flavonoids were the main groups of active substances, especially catechin, quercetin, resveratrol and epicatechin, while ferulic acid and gallic acid were the main phenolic acids. The extracts from all parts of black sugarcane showed tyrosinase inhibition activity which would be applied as an active ingredient in cosmetics for melasma protection. Antibacterial screening indicated the both small and big joint were the source of antibacterial substances. However, the phytochemical substances were suitable for other mechanisms of the human body like healthy supplements. This study could be suggested that the two black sugarcane cultivars are good sources of phytochemicals and antibacterial substances for use in health and cosmetics products.

SIGNIFICANCE STATEMENT

This study discovers the antipathogenic properties and antioxidant activity of black sugarcane (*Saccharum officinarum* L.). This study will help the researcher uncover the critical areas of using black sugarcane as an antipathogenic and antioxidant agents that many researchers were not able to explore thus, a new application using the black sugarcane as an antipathogenic and antioxidant agents may be arrived at.

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REFERENCES

1. del Río, J.C., G. Marques, A.G. Lino, C.F. Lima, J.L. Colodette and A. Gutiérrez, 2015. Lipophilic phytochemicals from sugarcane bagasse and straw. Ind. Crops Prod., 77: 992-1000.
2. Carvalho, M.J., A.L. Oliveira, S.S. Pedrosa, M. Pintado and A.R. Madureira, 2021. Potential of sugarcane extracts as cosmetic and skincare ingredients. Ind. Crops Prod., Vol. 169. 10.1016/j.indcrop.2021.113625.
3. Wczassek, L.R., S.D. Carlin, M.G. de Andrade Landell and M.T. Gamberini, 2022. Dopaminergic, cholinergic and nitrinergic pathways implicated in blood pressure lowering effects of *Saccharum officinarum* L. (sugarcane) on rats. Phytomed. Plus, Vol. 2. 10.1016/j.phyplu.2022.100238.
4. Dotaniya, M.L., S.C. Datta, D.R. Biswas, C.K. Dotaniya and B.L. Meena *et al.*, 2016. Use of sugarcane industrial by-products for improving sugarcane productivity and soil health. Int. J. Recyl. Org. Waste Agric., 5: 185-194.
5. Ji, J., M. Flavel, X. Yang, O.C.Y. Chen, L. Downey, C. Stough and B. Kitchen, 2020. A polyphenol rich sugarcane extract as a modulator for inflammation and neurological disorders. PharmaNutrition, Vol. 12. 10.1016/j.phanu.2020.100187.
6. Leal, M.R.L.V., A.S. Walter and J.E.A. Seabra, 2013. Sugarcane as an energy source. Biomass Convers. Biorefin., 3: 17-26.
7. Coutinho, I.D., J.M. Baker, J.L. Ward, M.H. Beale, S. Creste and A.J. Cavalheiro, 2016. Metabolite profiling of sugarcane genotypes and identification of flavonoid glycosides and phenolic acids. J. Agric. Food Chem. 64: 4198-4206.
8. Sun, J., X.M. He, M.M. Zhao, L. Li, C.B. Li and Y. Dong, 2014. Antioxidant and nitrite-scavenging capacities of phenolic compounds from sugarcane (*Saccharum officinarum* L.) tops. Molecules, 19: 13147-13160.
9. Asikin, Y., M. Takahashi, T. Mishima, M. Mizu, K. Takara and K. Wada, 2013. Antioxidant activity of sugarcane molasses against 2,2'-azobis(2-amidinopropane) dihydrochloride-induced peroxyl radicals. Food Chem., 141: 466-472.
10. Ali, S.E., Q. Yuan, S. Wang and M.A. Farag, 2021. More than sweet: A phytochemical and pharmacological review of sugarcane (*Saccharum officinarum* L.). Food Biosci., Vol. 44. 10.1016/j.fbio.2021.101431.
11. Ali, S.E., R.A. El Gedaily, A. Mocan, M.A. Farag and H.R. El-Seedi, 2019. Profiling metabolites and biological activities of sugarcane (*Saccharum officinarum* Linn.) juice and its product molasses *via* a multiplex metabolomics approach. Molecules, Vol. 24. 10.3390/molecules24050934.
12. Ji, J., X. Yang, M. Flavel, Z.P.-I. Shields and B. Kitchen, 2019. Antioxidant and anti-diabetic functions of a polyphenol-rich sugarcane extract. J. Am. Coll. Nutr., 38: 670-680.

13. Deseo, M.A., A. Elkins, S. Rochfort and B. Kitchen, 2020. Antioxidant activity and polyphenol composition of sugarcane molasses extract. Food Chem., Vol. 314. 10.1016/j.foodchem.2020.126180.
14. Feng, S., Z. Luo, Y. Zhang, Z. Zhong and B. Lu, 2014. Phytochemical contents and antioxidant capacities of different parts of two sugarcane (*Saccharum officinarum* L.) cultivars. Food Chem., 151: 452-458.
15. 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: 390-397.
16. Wang, L., P. Wang, W. Deng, J. Cai and J. Chen, 2019. Evaluation of aroma characteristics of sugarcane (*Saccharum officinarum* L.) juice using gas chromatography-mass spectrometry and electronic nose. LWT, 108: 400-406.
17. Kadam, U.S., S.B. Ghosh, S. De, P. Suprasanna, T.P.A. Devasagayam and V.A. Bapat, 2008. Antioxidant activity in sugarcane juice and its protective role against radiation induced DNA damage. Food Chem., 106: 1154-1160.
18. Abu Sufian, M., M. Rafiqul Islam, T.K. Chowdhury, Abdur Rahman, M. Sahab Uddin, S.F. Koly and M.S. Sarwar, 2017. Investigation of *in vivo* analgesic, anti-inflammatory, *in vitro* membrane stabilizing and thrombolytic activities of *Atylosia scarabaeoides* and *Crotalaria spectabilis* leaves. J. Pharmacol. Toxicol., 12: 120-128.
19. Al Mamun, A., Mahbubul Hossain, Ariful Islam, S. Zaman and M. Sahab Uddin, 2017. *Asparagus racemosus* Linn. potentiates the hypolipidemic and hepatoprotective activity of fenofibrate in alloxan-induced diabetic rats. Plant, 5: 1-12.
20. Abbas, Z.K., S. Saggu, M.I. Sakeran, N. Zidan, Hasibur Rehman and A.A. Ansari, 2015. Phytochemical, antioxidant and mineral composition of hydroalcoholic extract of chicory (*Cichorium intybus* L.) leaves. Saudi J. Biol. Sci., 22: 322-326.
21. Zahradníková, L., Š. Schmidt, Z. Sékelyová and S. Sekretár, 2008. Fractionation and identification of some phenolics extracted from evening primrose seed meal. Czech J. Food Sci., 26: 58-64.
22. Yilmazer-Musa, M., A.M. Griffith, A.J. Michels, E. Schneider and B. Frei, 2012. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. J. Agric. Food Chem., 60: 8924-8926.
23. Lavelli, V., P.S.C.S. Harsha, P. Ferranti, A. Scarafoni and S. Iametti, 2016. Grape skin phenolics as inhibitors of mammalian α-glucosidase and α-amylase-effect of food matrix and processing on efficacy. Food Funct., 7: 1655-1663.
24. El Hawary, S.S., M. Abubaker, E.M. Abd El-Kader and E.A. Mahrous, 2022. Phytochemical constituents and anti-tyrosinase activity of *Macadamia integrifolia* leaves extract. Nat. Prod. Res., 36: 1089-1094.
25. Joompang, A., P. Anwised, S. Klaynongsruang, L. Taemaitree and A. Wanthong *et al*., 2023. Rational design of an N-terminal cysteine-containing tetrapeptide that inhibits tyrosinase and evaluation of its mechanism of action. Curr. Res. Food Sci., Vol. 7. 10.1016/j.crfs.2023.100598.
26. Rattanasuk, S. and T. Phiwthong, 2021. A new potential source of anti-pathogenic bacterial substances from *Zamioculcas zamiifolia* (Lodd.) Engl. extracts. Pak. J. Biol. Sci., 24: 235-240.
27. Rattanasuk, S., R. Boongapim and T. Phiwthong, 2021. Antibacterial activity of *Cathormion umbellatum*. Bangladesh J. Pharmacol., 16: 91-95.
28. Rattanasuk, S., R. Boongapim, T. Phiwthong, S. Phuangsrik and N. Putthanach, 2021. Antibacterial profile of *Cissus quadrangularis* extracts against antibiotic-resistant bacteria isolated from Roi Et Hospital. Int. J. Pharmacol., 17: 97-102.
29. Zheng, R., S. Su, H. Zhou, H. Yan and J. Ye *et al*., 2017. Antioxidant/antihyperglycemic activity of phenolics from sugarcane (*Saccharum officinarum* L.) bagasse and identification by UHPLC-HR-TOFMS. Ind. Crops Prod., 101: 104-114.
30. Tanleque-Alberto, F., M. Juan-Borrás and I. Escriche, 2020. Antioxidant characteristics of honey from Mozambique based on specific flavonoids and phenolic acid compounds. J. Food Compos. Anal., Vol. 86. 10.1016/j.jfca.2019.103377.
31. Wu, D., J. Feng, M. Lai, J. Ouyang and D. Liao *et al*., 2020. Combined application of bud and leaf growth fertilizer improves leaf flavonoids yield of *Ginkgo biloba*. Ind. Crops Prod., Vol. 150. 10.1016/j.indcrop.2020.112379.
32. Chen, X.X., Y. Shi, W.M. Chai, H.L. Feng, J.X. Zhuang and Q.X. Chen, 2014. Condensed tannins from *Ficus virens* as tyrosinase inhibitors: Structure, inhibitory activity and molecular mechanism. PLoS ONE, Vol. 9. 10.1371/journal.pone.0091809.
33. Helen, L.R., M. Jyothilakshmi and M.S. Latha, 2015. Isolation and quantification of tannins from the root bark of *Clerodendrum infortunatum* Linn. and assessment of their antioxidant potential and antiproliferative effect on HCT-15 cells. Int. J. Pharm. Sci., 7: 170-175.
34. Zheng, R., S. Su, J. Li, Z. Zhao, J. Wei, X. Fu and R.H. Liu, 2017. Recovery of phenolics from the ethanolic extract of sugarcane (*Saccharum officinarum* L.) baggase and evaluation of the antioxidant and antiproliferative activities. Ind. Crops Prod., 107: 360-369.
35. Katalinić, V., S.S. Možina, D. Skroza, I. Generalić and H. Abramovič *et al*., 2010. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). Food Chem., 119: 715-723.

36. Meng, J.F., Y.L. Fang, M.Y. Qin, X.F. Zhuang and Z.W. Zhang, 2012. Varietal differences among the phenolic profiles and antioxidant properties of four cultivars of spine grape (*Vitis davidii* Foex) in Chongyi County (China). Food Chem., 134: 2049-2056.
37. Chen, Y.H., Z.S. Yang, C.C. Wen, Y.S. Chang, B.C. Wang, C.A. Hsiao and T.L. Shih, 2012. Evaluation of the structure-activity relationship of flavonoids as antioxidants and toxicants of zebrafish larvae. Food Chem., 134: 717-724.
38. Shamsul Ola, M., M.M. Ahmed, R. Ahmad, H.M. Abuohashish, S.S. Al-Rejaie and A.S. Alhomida, 2015. Neuroprotective effects of rutin in streptozotocin-induced diabetic rat retina. J. Mol. Neurosci., 56: 440-448.
39. Li, X., J. Lin, Y. Gao, W. Han and D. Chen, 2012. Antioxidant activity and mechanism of *Rhizoma cimicifugae*. Chem. Cent. J., Vol. 6. 10.1186/1752-153X-6-140.
40. Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Byrne, 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal., 19: 669-675.
41. Antoniolli, A., A.R. Fontana, P. Piccoli and R. Bottini, 2015. Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. Food Chem., 178: 172-178.
42. Motham, P., A. Thonpho and P. Srihanam, 2021. Antioxidative compounds investigation in sugarcane bagasse extracts fractionated by silica gel column chromatography. Asian J. Plant Sci., 20: 601-608.
43. Burin, V.M., N.E. Ferreira-Lima, C.P. Panceri and M.T. Bordignon-Luiz, 2014. Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: Evaluation of different extraction methods. Microchem. J., 114: 155-163.
44. Lou, S.N., M.W. Yu and C.T. Ho, 2012. Tyrosinase inhibitory components of immature calamondin peel. Food Chem., 135: 1091-1096.
45. Huang, X.X., Q.B. Liu, J. Wu, L.H. Yu and Q. Cong *et al*., 2014. Antioxidant and tyrosinase inhibitory effects of neolignan glycosides from *Crataegus pinnatifida* seeds. Planta Med., 80: 1732-1738.
46. Zhao, Y., M. Chen, Z. Zhao and S. Yu, 2015. The antibiotic activity and mechanisms of sugarcane (*Saccharum officinarum* L.) bagasse extract against food-borne pathogens. Food Chem., 185: 112-118.
47. Williams, I.O., E.O. Onyenweaku and I.J. Atangwho, 2016. Nutritional and antimicrobial evaluation of *Saccharum officinarum* consumed in Calabar, Nigeria. Afr. J. Biotechnol., 15: 1789-1795.
48. Amalia, A.V., K.K. Pukan, N. Setyawati, T. Widiatningrum and U. Khasanah, 2019. Antibacterial activity of *Saccharum officinarum* leaves extract against food-borne disease. J. Phys.: Conf. Ser., Vol. 1321. 10.1088/1742-6596/1321/3/032043.
49. Uchenna, E., O. Adaeze and A. Steve, 2015. Phytochemical and antimicrobial properties of the aqueous ethanolic extract of *Saccharum officinarum* (sugarcane) bark. J. Agric. Sci., 7: 291-297.