Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(2): 391–398

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Comparative Phytochemical Screening and Biological Activity of "Lyophilized Sugarcane Juice, Vacuum Pan and Open Pan Jaggery" for Methanolic Extract

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Received: 09 Jan 2021 | Revised accepted: 16 Feb 2021 | Published online: 02 Mar 2021

A B S T R A C T

Sugarcane jaggery contains many industrially important phytochemicals. To study the difference and characterize in phytochemicals present in lyophilized sugarcane products methanolic extracts of sugarcane juice (SJ), vacuum pan jaggery (VJ), and open pan jaggery (OJ) and their biological activity like antioxidant activity and antimicrobial activity against certain clinical isolates were estimated, Antioxidant activities were evaluated by ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) DPPH (1,1 diphenyl 2, picryl hydrazyl) assay and Reducing Power. Various pathogenic strains were used to determine the antimicrobial activity where the samples showed promising inhibition of such organisms. Reference should be made to the fact that our work constitutes the first analysis to test the vacuum evaporated jaggery substance as a possible antioxidant resource to be explored in vitro in cellular systems.

***Key words*:** Lyophilized Jaggery, Vaccum pan jaggery, Phytochemicals, Antioxidants, Phenols, Flavonoids

In India, the most common sweetener is called jaggery, which is prepared by concentrating the sugarcane juice extracted. It is eaten by all parts of society as a sweetener cum energy source, since it is the healthiest natural sweetener containing minerals and vitamins [1]. Sugarcane (*S. officinarum*) is used in several areas of Asia as a traditional medicine to treat various liver diseases, hemorrhoids, and dyspepsia [2] and it is known that oxidative damage is involved in many human diseases such as cancer, cardiovascular diseases or other degenerative disorders. Phytochemicals such as phenolics present in sugarcane juice are believed to have antibacterial, antiviral and antiseptic activities. Ayurvedic drugs indicate that the use of sugarcane root and stem plays an important role in the treatment of skin problems, urinary tract infections (UTIs), bronchitis, cardiovascular disease, coughing, anemia and absence of breast milk [3]. The survival rate of polyphenols and other phytochemicals in the microbial strain depends on the strains of bacteria, phenolic composition and compound concentration [4]. The most prevalent complexes in plants that have antioxidant function by their redox response are phenolic compounds [5]. The scientific interest in phenols and policosanols (PC) present in Jaggery has received worldwide attention as the compounds exhibit great biological activities and health benefits. Policosanols (PCs) consist of primary long-chain alcohols of differing chain lengths, ranging from 22 to 34 carbon atoms, and display lipids or cholesterol-reducing activity [6]. Differences in processing, as well as in the origin and sugarcane cultivar, could be responsible for these discrepancies [3]. It is mentioned that the least refined Jaggery preserves most of the phytochemicals that are found in sugar cane juice [7]. Studies also indicated that sugarcane extract's anti-microbial potential performs better in inhibiting *P. aeruginosa* relative to *S. Auroraeus* [8]. Vacuum drying provides an ability to counter any adverse results and increase the quality and nutrient benefits of a product [9] when dried in a reduced-pressure environment. Therefore, the vacuum drying technique may enhance the features of palm jaggery sugar [10-11].

The heat treatment influence was heterogeneous and the effects were indeed based on the sugar cane product being analyzed, and also on the method of testing used. The antioxidant function of the products did not change and even increase based on the combination of temperature and time. Cooking and other therapies have thus been documented in order to improve and in other cases reduce the Antioxidant activity potential of such vegetables [12]. As per the research findings, shorter durations and low to medium temperatures

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might enable the reduction of antioxidants naturally present in the samples, while a decrease in processing would contribute to the formation of other or more active compounds. Maillard reaction products may have existed in the case of sugarcane products and, thus, have created an improvement in Antioxidant activity ability. Increased bioavailability of antioxidants or increased activity of naturally occurring antioxidants could also occur as a consequence of heating procedure [13]. In the other hand, variations may be clarified by their distinct exposure to the multiple antioxidant compounds present in the sample due to the assay procedure used. Even as statistically significant variations were observed for certain items, such as brown sugar and cane honey, there was no serious change in the Antioxidant activity capability due to thermal treatment. In fact, Antioxidant activity assets of jaggeries were not impacted. Food processing will, as mentioned, enhance the property of natural antioxidants or encourage the development of new antioxidant-capable compounds, so that the total antioxidant activity can increase or maintain unchanged [13]. In the specific case of non-refined sugarcane products, it can therefore be inferred that processing at temperatures below 100°C and periods below 60 min will not have a substantial effect on their antioxidant property [14]. The detection, separation, and purification of certain bioactive pharmaceutical ingredients is growing day by day due to the existence of many relevant and significant bioactive compounds. These attractive features of sugar cane juice and its derivatives have prompted researchers. However, no report on differences in the phytochemicals and biological activity of sugarcane jaggery generated under the vacuum evaporation process is currently available. Consequently, comparative analysis of different phytochemicals (phenolic content, flavonoid content, tanin content, saponin content, alkolide content, terponide content) and protein content of lyophilized sugarcane products (juice, vacuum pan and open pan jaggery) present in methanolic extract was conducted in this study to assess health benefits and other bioactivities (ABTS, DPPH, ferric reducing power) and antimicrobial properties of these products.

MATERIALS AND METHODS

Chemicals: In this particular study number of chemicals were used for different assays. were purchased from Himedia Laboratories, Mumbai, India. Other chemicals which were used for sample extraction, confirmation test, and various assays were obtained from local suppliers of chemicals, Bangalore.

Collection of sugarcane variety and juice extraction

The VCF-0517 sugarcane sample was taken from the V.C. zone agricultural research station. Mandya, Mysore, Karnataka, India. Between the months of June and July 2019. The sugar cane was washed with distilled water and the outer skin and dirt was removed using a clean, sterile knife and the fresh sample weight was noted. The sugar cane juice was extracted using a clean crusher to avoid contamination of any kind from previous debris. Sugarcane bagasse was weighed after extraction. The sugar cane juice sample was used as raw materials for lyophilization preparation of juice, open pan and vacuum pan jaggery extractions.

Preparation of the lyophilized samples and extraction

After extracting juice and filtrating the pH had been changed to 7 (only for vacuum pan jaggery and open pan jaggery), the juice was boiled to a maximum of 115°C and condensed to a solid shape, then swirling cooling until solid jaggery was shaped. The Jaggery sample of vacuum juice was evaporated using a 65°C vacuum evaporator and vacuum pressure was set at 120 psi. The tow sample were lyophilized and processed for further use in a deep freezer. For preparing the sucre cane juice sample, the sugar cane juice had been filtered, frozen and stored at -20°C before further application and samples were frozen and stored. The row sugar cane was collected and the juice was extracted immediately before sample preparation. One hundred grams of each sample was mixed in 500 ml of deionized water and then shook at 500 rpm for 24 hours. The extract was filtered with a sterile Whatman paper filter grade 1 and put in a refrigerator during further use [15]. To concentrate the samples, the raw extract was evaporated by distillation to one-fourth of the original sample volume. Concentrated samples have been stored under refrigeration for further study.

Physico-chemical characterization

pH: The determination of pH was done using Comsys Digital pH meter.

Water Activity aw: The instrument used to measure water activity was Capacitance or Electric Hygrometers.

Moisture content: Moisture analysis was done by the Oven Drying Method or the Loss on drying method as per [16] using CHEMI Digital Hot Air Oven.

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100$$

Phytochemical analysis

Qualitative assays: The qualitative phytochemical analysis was performed in triplicates of the samples following the protocol mentioned by [17].

Quantitative assays: The total phytochemical content for each compound was determined by quantitative assays for all the samples.

Antioxidant assay: ABTS radical scavenging was performed for estimation of the antioxidant capacity following the procedure described by [18]. On the basis of the following equation, the percent inhibition of ABTS capacity was calculated:

$$I\% = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

By plotting the sample concentration implemented (as phenol concentration mg/ml) against the corresponding scavenging activity %, the IC50 value (concentration providing 50% inhibition) was determined.

DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by a free radical scavenger [19]. Based on the following equation, the inhibition of DPPH ability was calculated:

$$I\% = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

The IC50 value was measured against the corresponding scavenging activity % by plotting the implemented sample concentration (as phenol concentration mg/ml) as well.

The reducing power assay of the phytochemicals in the methanolic extract was performed following the protocol

mentioned by [20-21] higher absorbance indicates higher reducing power.

Antimicrobial Activity

Antimicrobial activity of the methanolic extract of SJ, VJ, and OJ samples was performed by Agar Well diffusion method [22] using Muller Hinton Agar (MHA- Hi-Media) against 10 different clinical isolates. 80µl of sample was added in each well along with Methanol as control.

RESULTS AND DISCUSSION

Physico-chemical characterization

Determination of pH: The pH of sugarcane juice was measured before the preparation of all samples and result after lyophilization only showed in (Table 1). Upper pH for jaggery extracts due to the addition of lime to modify the pH. The drop in acidity induced a concomitant rise in the pH value suggests that the pH value influenced by the variance of the jaggery phase as the VJ and OJ samples were always deemed to be a low acid product because their pH level was more above 6, which agreed with the results reported by [23]. As prescribed by [24] for panela which established minimum pH of 5.90 that supports the results reported by [25]. pH reduction was reported following heat treatment on sugarcane juice, these observations highlighted that non-thermal treatments might have milder effects on the physicochemical properties of the samples, as it is less intrusive than heat [26]. The pH of crystal sugars is greater than that of jaggery and cane honey. In the sugar production process, carbonation takes place, which enables the formation of unwanted impurities and raises the pH of the syrup. The discrepancy in pH can also be attributable to other compounds such as organic acids and the use of citric acid as a preservative in the case of CH [14].

Table 1 Physico-chemical characteristics of SJ, VJ and OJ after Lyophilization

Sample name	pH	Water activity aW	Moisture content %
SJ	5.15±0.026 c	0.3669±0.013 b	4.72±0.51 b
VJ	6.36±0.025 a	0.4742±0.010 a	5.93±0.09 a
OJ	6.13±0.036 b	0.4835±0.015 a	3.52±0.22 c

Values are expressed as mean ±SD (n = 3)

Different letters in the same columns indicate significant difference among means ($p < 0.05$)

Water activity (aw): is one of the key causes why jaggery or sugar cane products are ruined in long-term. For each finding obtained differed (Table 1). The findings indicate that water activity is sufficiently limited for microorganisms to avoid the development and spoilage, as three samples have shown less water activity than the ideal range for microorganism growth [23].

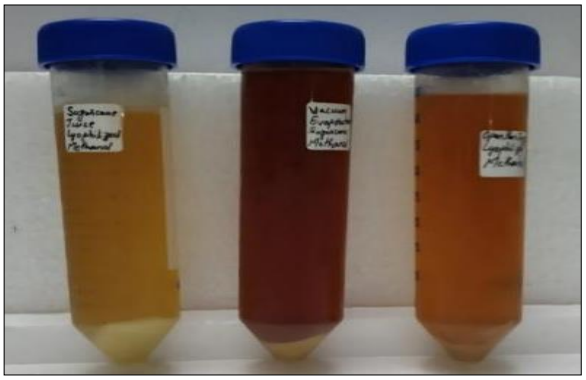


Fig 1 Methanolic extracts of SJ, VJ and OJ samples

Moisture content: The high the moisture, the lesser the usable life or is liable to spoilage due to contamination by microorganisms, the water content determines the storage life of any product. The Indian Standards Bureau, BIS 1990, sets out 5–7 per cent of fresh jagger moisture [27]. The moisture content was found to be the least in lyophilized open pan jaggery (3.52%) followed by lyophilized sugarcane juice (4.72%) and lyophilized vacuum pan Jaggery (5.93%) (Table 1). The processing of the jaggery and crystallization thereafter lower the moisture content and hence improves the shelf life. After lyophilization, it was observed that the sugarcane juice and vacuum pan jaggery was highly hygroscopic and absorbed moisture coming in contact with atmospheric air and hence the increase in the moisture content. There is a study indicated that the hygroscopicity is not only a function of temperature but also depends on the water activity, the crystal size of the sample, and the other components of the food [28]. As presented in another report, the physical parameters (water content, aw, pH, color) of palm granulated sugar. The water content of studied palms differed from 2.91 percent to 5.12 percent. The water content of 100 C drying samples was higher than 80 C and 90 C and was also close to previous palm sugar studies (0.98% - 2.47%) [11]. The water content variation of sugars is created by differences in the manufacturing process [29]. The aw values of the palm granulated sugar ranged from 0.48 to 0.30. The lowest and highest aw values were for samples OPS1 (100°C, 90 min) and EPS1 (80°C, 60 min). The high aw values quickly promote microbial growth and biochemical degradation reactions, all of which shorten the storage of sugars [23]. These results showed that palm granulated sugars could extend the storage time, and the OPS1 sample was the most stable. This result agreed with an earlier report on water activity [11]. The pH values were modified slightly, from 6.90 to 6.99. Our data were higher than the results of previous studies in which granulated jaggery had pH values of 5.26 and 6.60 [30]. The pH value change may be explained by chemical reactions occurring during the palm jaggery heating process [31] and by the appearance of Maillard reaction products (MRPs) [32]. Although water activity was relatively similar for all samples, water content was significantly higher for cane honey than for jaggeries and crystal sugars. Jaggeries water content was in the range of the reported by other authors was reported by [33].

Table 2 Qualitative phytochemical analysis of methanolic extracts of SJ, VJ and OJ

Sample/Compounds	SJ	VJ	OJ
Phenols	+++	+++	+++
Flavonoids	+	+	++
Tannins	+++	+++	+++
Saponins	+	+	+
Alkaloids	++	++	++
Terpenoids	++	++	+++
Proteins	++	++	++

+++(Much abundant), ++(Less abundant), +(Minute), - (Absent)

Phytochemical were determining with the methanolic extract of SJ, VJ, and OJ samples. (Table 2) shows the presence of some phytochemicals such as phenols, flavonoids, tannins, saponins, alkaloids, terpenoids, and proteins, in all the three experimented samples.

Quantitative analysis

TPC is expressed as Gallic acid, TFC is expressed as catechin, TC is expressed as Linalool, SC is expressed as saponin, TAC is expressed as Alkaloid, TTC is expressed as Tannic acid. PC is expressed as Bovine Serum Albumin. The mean values and standard deviation for total phytochemicals such as TPC, TFC, TC, TTC and PC were calculated from a calibration curve of each (Fig 2-6).

Table 3 Quantitative phytochemical analysis extracts of SJ, VJ and OJ (mg/ml)

Sample	TPC	TFC	TC	SC	TAC	TTC	PC
SJ	2.39±0.90 b	2.35±0.30 a	46.47±1.90 a	48±11.31 a	520±16.00 a	141.65±11.11 c	1.0±0.20 a
VJ	2.88±0.12 ab	2.43±0.17 a	50.43±2.80 a	44±4.00 a	440±44.00 b	277.40±8.15 a	0.7±0.20 ab
OJ	3.74±0.52 a	2.01±0.51 a	48.41±0.80 a	48±6.00 a	180±20.00 c	258.70±5.13 b	0.5±0.10 b

Values are expressed as mean ±SD (n = 3); Different letters in the same columns indicate significant difference among means ($p < 0.05$)

The amount of total phenolic contents of the mean values of phenol content of Si, Vi and OJ are given in (Table 3) there were significantly higher in OJ which was 3.74 mg GAE/ml compared with VJ and SJ which were 2.88 and 2.39 mg GAE/ml respectively. Regarding the processing effect on total phenolic content, the results in this study are in accordance with the previous finding reported by [34] differed among samples from 2.77 to 8.94 mg/100 g. At 80°C, the total phenolic content was the highest from 7.55 to 8.94 mg/100 g. When the temperature increased to 90°C and 100°C, the total phenolic contents were significantly increased from 4.64 to 7.62 mg/100 g and from 2.77 to 3.13 mg/100 g, respectively. Phenolic content is easily destroyed during the heating process. Similar findings reported that the total phenolic content of palm sugar ranged from 2.14 to 16.29 mg/100 g [11] and 0.48 µg of GAE/mg. The interference of the sucrose, fructose and glucose was more significant when added to standard of gallic acid curves which led to increasing in the total phenol content of sugar can products [14]. Total flavonoid content for VJ and OJ were 2.43 and 2.01mg Catechin /ml, respectively and for sugarcane juice 2.351. The results of the present study (Table 3) were not indicating high significance in the mean value of total flavonoid content between the different samples for VJ, OJ and SJ. Previous studies reported that the sugarcane is rich in flavonoid compounds, and values for total flavonoids were observed to be higher than values for total phenols. Since quercetin has a maximum absorption at 445 nm, although some of the flavones abundant in sugarcane (tricine and apigenin) have a maximum absorption below 400 nm when reacted with AlCl3, the overall flavonoid content could be overestimated by calculating the absorbance of the reaction at 368 nm using quercetin as a norm. On the counter, as has already been indicated, calculating the absorbance above 400 nm will underestimate the flavonoid material. even in the literature, this colorimetric approach is widely used to approximate the overall flavonoid content without taking these details in account [14]. Note that in this research we used 415 nm to determine the flavonoid content. The mean values of tannin content were not increased significantly as presented in (Table 3). Saponin content values as shown in (Table 3) were not significantly different between different samples. The mean values of total alkaloid content in the extracted samples were significantly different between different samples which were 520, 440, and 180 mg/ml for SJ, VJ and OJ respectively. The results showed in table 3 a highly significant increase in the content of terpenoid for VJ, OJ and SJ which were 277.40, 258.70 and 141.65 Linalool equivalent mg/ml respectively. The mean values of protein content were significantly different between the samples as presented in (Table 3) which were 1.0, 0.7 and 0.5 mg/ml for SJ, VJ and OJ BSA/ml respectively (Table 3).

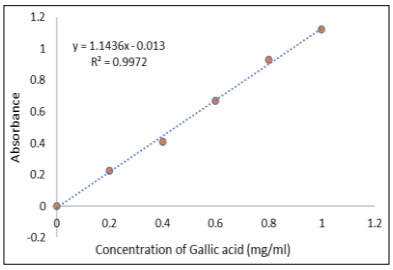


Fig 2 gallic acid standard curve

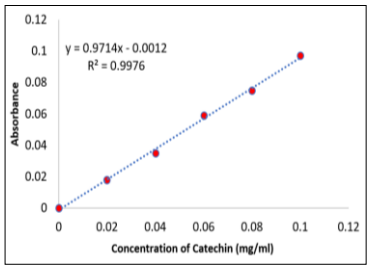


Fig 3 catechin standard curve

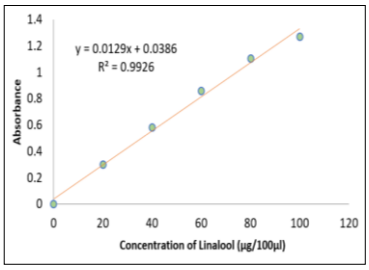


Fig 4 linalool standard curve

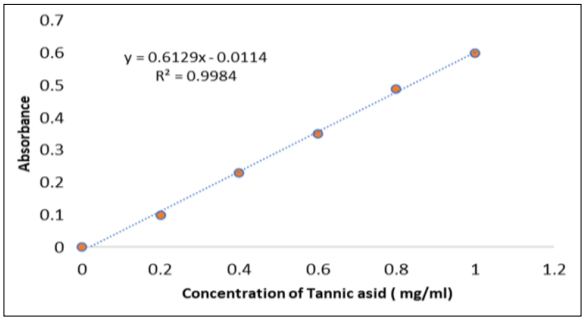


Fig 5 Tanic acid standard curve

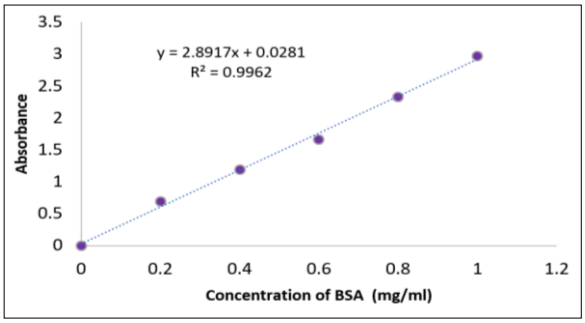


Fig 6 BSA standard curve

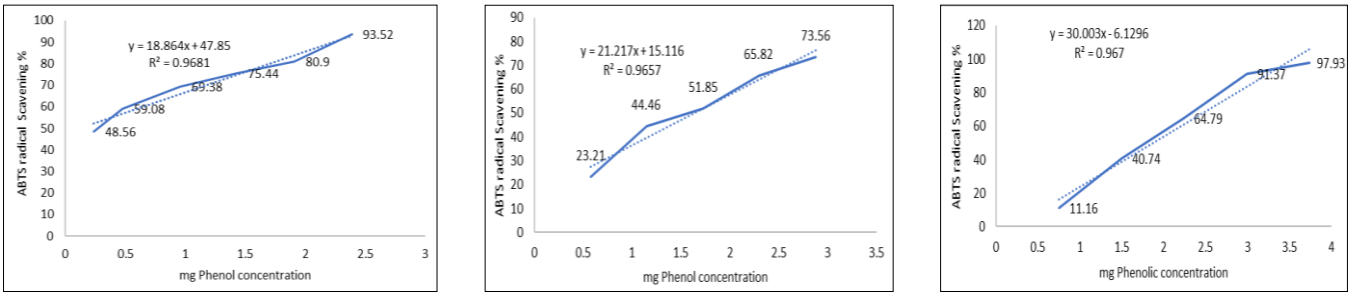
The standard curve of concentration with increases O.D values are presented as means ± SD (n = 3)

Antioxidant assay

The Antioxidant assay of SJ, VJ, and OJ methanolic extract was estimated by three in-vitro assays, ABTS, DPPH, and Reducing Power assay. It is recommended the use of more than one single method to estimate the antioxidant activity of complex samples [35]. The ABTS free radical method, which has been reported to be more sensitive to hydrophilic antiradical [36], was used in addition to DPPH˙ radical scavenging capacity.

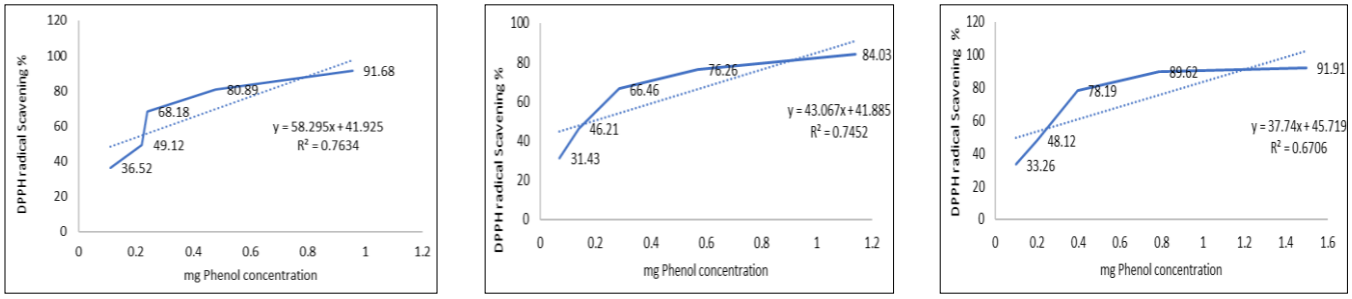
ABTS [*2,2'-azinobis* (*3-ethylbenzothiazoline-6-sulfonic acid*)]

The antioxidant capacity of SJ, VJ and OJ were plotted as antioxidant effect of gallic acid equivalent in a line diagram. This diagram depicts a positive trend as the scavenging activity increases with the increasing mg gallic acid equivalent of the sample. The OJ shows the highest antioxidant properties 97.93%. While SJ shows a steady increase in the antioxidant activity with the increasing concentration but most importantly, the VJ sample shows moderate antioxidant capacity (73.56%) as compared to the SJ shows high antioxidant property (93.52% activity) R^2 is the correlation coefficient between phenolic contents and ABTS scavenging activity % for each which indicates strong correlation in this study (Fig 7). Phenolic compounds, primarily flavonoids, phenolic acids, and polyphenols, have been linked to the antioxidant properties of sugar cane. Phenolic food compounds, particularly flavonoids, are considered to play an important role in human health [37]. The antioxidant properties of sugarcane juice could partly explain its medicinal effects [3]. Several studies have documented a link between phenolic content and antioxidant activity [38-39]. The phenolic content regulates the antioxidant potential through different pathways related to free radical scavenging [40].



SJ Sample VJ Sample OJ Sample

Fig 7 ABTS inhibition radical of SJ, VJ, OJ Samples



SJ Sample VJ Sample OJ Sample

Fig 8 DPPH Inhibition Radical of SJ, VJ, OJ samples

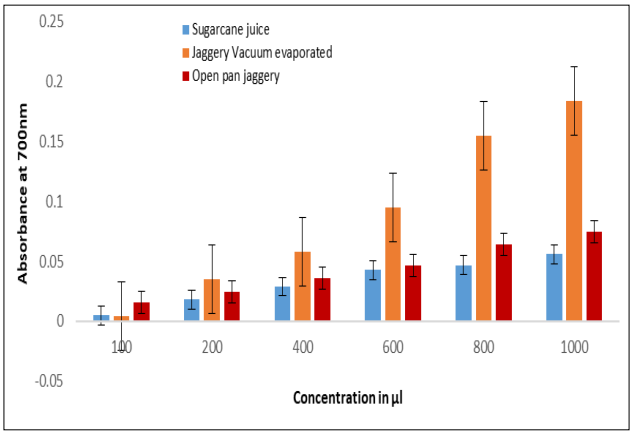
DPPH Assay (1,1-Diphenyl- 2- picryl hydrazyl) [41]

In this DPPH (1,1-Diphenyl- 2- picryl hydrazyl) assay all three samples are showing a positive trend as the scavenging activity increases with increasing concentration. Moreover, unlike ABTS assay VJ shows high antioxidant activity (84.03%) as compared to other samples and the open pan jaggery shows the highest scavenging ability (91.91% for OJ whereas 91.68% for SJ). The correlation coefficient R^2between DPPH scavenging activity % and phenolic contents indicates good correlation in this study for three samples (Fig 8). As reported by (Py2005) in an analysis of the DPPH radical scavenging activity of cane brown sugar, a similar trend was observed, from 14.5% to 26.90% and granulated non-centrifugal sugars, from 38.04% to 71.08%. Grown MRPs and caramelization products may be the result of the growing DPPH radical scavenging activity. Previous experiments have shown that MRPs are able to donate hydrogen and have the ability for free radical reactions [42]. Palm granulated sugar's potential antioxidant effects were anticipated by its ability to minimize the TPTZ-Fe(III) complex to the TPTZ-Fe(II) complex [43-44] in the other study results were obtained with the DPPH test, as brown sugars had the lowest potential for antioxidant activity, and the highest were light jaggeries and cane honey [14]. Differences between the methods of DPPH and TEAC-ABTS is mostly due to their differing susceptibility to the antiradical compounds that could be found in the products of sugar cane. There were also some differences in reaction time; however, the reaction of ABTS is generally faster than the reaction of DPPH inhibition [35].

Reducing power assay [20]

A comparison of SJ, VJ and OJ samples was shown in (Fig 9) as a trend in their reducing power. As the result indicate, the lower concentration of the samples did not show any activity but as the concentration of the sample increased significantly it exhibited a higher activity samples with VJ, OJ and SJ in all the various concentrations respectively, an increase in the concentration of extracts led to a significant increase ($p \leq 0.05$) in the absorbance values (higher reducing power). The methanolic extract of each sample was tested for the oxygen scavenging and reducing ability. There is no doubt that VJ retains much of phytochemicals than OJ. Similar results were found for the FRAP value of raw cane sugar was 0.17 to 0.33 mmol/100 g, that of dark brown sugar was 0.69 mmol/100 g and that of granulated white sugar was 0.01–0.02

mmol/100 g, which were lower values than the present results [45]. The alterations in the antioxidant potential of unrefined sugars were determined by factors such as the methods used for preventing antioxidant effectiveness [46]; the ratio of inverted sugars; the amount of phenolics, flavonoids [14] and processing methods [47]. Another element is the concomitant production of Fe(II), which is a known pro-oxidant and can bring about the production of additional radicals in the reaction medium (such as OH• from the Fenton reaction). Then, the absorbance of these compounds was measured, leading to falsely high results for the FRAP value [48].



Values are expressed as mean ± SD (n = 3); statical analysis for significant difference among means ($p < 0.05$)

Fig 9 Reducing power capacity for SJ, VJ and OJ samples at different concentrations extract

Table 4 IC50 values for extract samples

Sample	IC50 values for ABTS assay	IC50 values for DPPH assay
SJ	1.87±0.19^{a}	0.14±0.01^{b}
VJ	1.64±0.16^{a}	0.19±0.02^{a}
OJ	0.11±0.01^{b}	0.11±0.01^{c}

Values are expressed as mean ± SD (n = 3)
Different letters in the same columns indicate significant difference among means ($p < 0.05$)

IC50 ABTS and DPPH Assay

The comparative (Table 4) of the different antioxidant assays showed different IC50 values. For ABTS assay VJ showed higher antioxidant activity than OJ followed by SJ, But for DPPH assay OJ showed higher antioctioxidant activity followed by SJ. This IC50 parameter has the drawback that as greater an antioxidant activity, the smaller its IC50 value [49]. Results indicated that IC50 values for ABTS assay in (Table 4) showed the highest IC50 was observed for SJ followed VJ while the lowest was in OJ significantly which were 0.11 mg phenolic concentrations/ml sample extract, 1.64 and 1.87 respectively. Whereas IC50 values for DPPH assay the mean values showed in (Table 4) for OJ, SJ and VJ which were 0.11, 0.14 and 0.19 mg phenolic concentrations/ml sample extract respectively. Other results reveal that cane honey has an IC50 of 1.31 g product/mL and 2.5 g product/mL of granulated jaggery.

Antimicrobial activity [22]

The antimicrobial activities of the methanolic extracts of SJ, VJ, and OJ were evaluated against several pathogenic bacteria using the agar diffusion assay method. Selected pathogenic bacteria have belonged to Gram-positive bacteria, including *Staphylococcus aureus, Enterococcus faecalis, Candida albicans, Bacillus subtilis Streptococcus mutans,* and some other have belonged to Gram-negative bacteria, including *Proteus Vulgaris, Klebsiella pneumonia, Salmonella typhi, Escherichia coli,* and *Pseudomonas aeruginosa.* In general, SJ methanolic extract showed more significant inhibition against *Staphylococcus aureus* (10.3 mm), *Proteus Vulgaris* (5.7 mm), *Enterococcus faecalis* (9.0 mm), and *Bacillus subtilis* (8.7 mm). While, showed no inhibition effect against *Candida albicans* and *Salmonella typhi,* compared to the other two methanolic extracts (i.e., VJ and OJ) (Table 5). On the other hand, the VJ methanolic extract showed higher inhibition against *Candida albicans* (2.7 mm), *Salmonella typhi* (2.3 mm), and *Streptococcus mutans* (4.7 mm) than the other two extracts (i.e., SJ and OJ). In contrast, no inhibitions were being shown against three pathogenic bacteria, *Proteus Vulgaris, Escherichia coli,* and *Pseudomonas aeruginosa* (Table 5).

Table 5 Antimicrobial activities of SJ, VJ, OJ in terms of forming zone of inhibition

Sample	Con. of samples	*Staphylococcus aureus*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Enterococcus Faecalis*	*Candida albicans*	*Salmonella typhi*	*Bacillus subtilis*	*Streptococcus mutans*	*E. coli*	*Pseudomonas aeruginosa*
SJ	0.951g/m	10.33±2.51^{a}	5.66±4.93^{a}	8.00±1.00^{a}	9.00±2.00^{a}	0±0^{a}	0±0^{a}	8.66±0.57^{a}	2.66±4.61^{a}	5.33±4.77^{ab}	9.33±0.57^{b}
VJ	0.933g/m	9.33±1.52^{a}	0±0^{a}	8 .66±1.15^{a}	7.66±0.57^{a}	2.33±4.04^{a}	0±0^{a}	2.66±4.61^{a}	2.33±4.04^{a}	7.66±1.15^{a}	10.33±0.57^{a}
OJ	0.954g/m	2.66±4.61^{b}	0±0^{a}	5.00±4.35^{a}	8.66±0.57^{a}	2.66±4.61^{a}	2.33±4.04^{a}	3.00±5.19^{a}	4.66±4.04^{a}	0±0^{b}	0±0^{c}
	Methanol (C)	8±0	2.3±2.3	5.3±2.7	2.3±2.3	6.3±3.2	0±0	2.3±2.3	0±0	0±0	8.3±0.3

Values are expressed as mean ±SD (n = 3)
Different letters in the same columns indicate significant difference among means ($p < 0.05$)

The OJ methanolic extract showed significant inhibition against *Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa,* compared to other extracts (Table 5). Gram-positive or Gram-negative bacteria were both affected by methanolic extracts (Table 5). *Staphylococcus aureus* and *Enterococcus faecalis* were the highest inhibited bacteria among the Gram-positive bacteria, while *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were the highest affected bacteria among the Gram-negative [50]. They noted that the antibacterial inhibition of sugarcane molasses extract showed more significant inhibition against Gram-positive bacteria than Gram-negative bacteria. Also, our findings were similar to [51] in regard to inhibition of Gram-negative bacteria, which they stated that Gram-negative bacteria was inhibited more easily by the molasses extracts. In fact, the variation on the obtained inhibition could not be only regarded to the differences of Gram-positive or Gram-negative, but also can be attributes to the differences in susceptibility of each

type of bacterium to the antibacterial substances such as polyphenols and antioxidants chemicals. In the antimicrobial testing of the samples, the organisms showed significant differences between the samples in *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* were not shown significantly different in *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterococcus Faecalis*, *Candida albicans*, *Salmonella typhi*, *Bacillus Subtilis* and *Streptococcus mutans* as presented in (Table 5).

CONCLUSIONS

The result of this study explains the presence of number of different phytochemicals with health and industrial benefits. Due to presence of antioxidants in sugarcane juice and its products these are becoming nutraceutically important. According to this study sugarcane juice contains large number of antioxidants and vacuum pan jaggery also contains moderate number of different important phytochemicals. The sugarcane juice and jaggery also show the presence of antimicrobial agents. It can be stated that consumption of sugarcane juice and jaggery can help in improving body metabolisms and other minor health conditions. As the use of sugarcane and jaggery nowadays getting in industrial interest, more research to be done to categorize the phytochemicals and their efficacy for utilizing them as neutraceuticals and biomedicines in near future.

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