ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

EVALUATION OF PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITY OF LYOPHILIZED SUGARCANE JUICE, VACUUM AND OPEN PAN JAGGERY FOR AQUEOUS EXTRACTS

Muneer Saif Hasan Ahmed[1], Harish Nayaka MA*[2], Yaaser Q. Almulaiky[3,4], Sam Al-Dalali[5]

[1]*Department of Studies in Biochemistry, University of Mysore, Mysore, India*

[2]*Department of Sugar Technology, Sir. M. Visvesvaraya Post Graduate Centre, University of Mysore, Mandya, India*

[3]*University of Jeddah, College of Sciences and Arts, Khulis, Department of Chemistry, Jeddah, KSA*

[4]*Chemistry Department, Faculty of Applied Science, Taiz University, Taiz, Yemen*

[5]*Department of Food Science and Technology, Ibb University, Ibb 70270, Yemen*

**Corresponding author: harish_nayaka4@yahoo.com*

ABSTRACT

Lyophilized aqueous extracts of sugarcane juice (SJ), vacuum pan jaggery (VJ), and open pan jaggery (OJ) were estimated for their Physico-chemical properties, phytochemical constituents, and biological activity against certain clinical isolates. The phytochemical results of flavonoid and tannin content indicated high significance in the mean values of the VJ sample whereas, phenol and terpenoid content was significantly higher in the OJ sample. For antioxidant activity, the mean values of IC_{50} of DPPH assay and ABTS assay showed a highly significant increase for OJ, which were 2.810 and 2.441% respectively. All the aqueous extract showed a zone of inhibition against tested organism extract except *Salmonella typhi. Bacillus subtilis,* and *Pseudomonas aeruginosa*. The results indicate that the Lyophilized SJ, VJ and OJ have phytochemical components that can have other clinical and medicinal properties that use these products not only as a food additive but as pharmaceutical additives. Worth a mention that our study constitutes the first research to evaluate vacuum evaporated jaggery product as a potential antioxidant resource to investigate by *in vitro* trials in cellular systems.

Keywords: Vacuum pan jaggery, Open pan jaggery, Phytochemical, Antioxidants, Antimicrobial activity.

1. INTRODUCTION

India is one of the top five producers worldwide of sugar cane [1], and the largest producer and consumer of jaggery, contribute to over 70% of the world's production [2]. In India, the jaggery and Khansari sectors use about 19.1 per cent of sugar cane, that supplies around 7 million tons of jaggery a year [3]. Jaggery contains sucrose 80-85 %, reducing sugars involves both fructose and glucose10-15 %, fat 0.25 %, and minerals 0.6-1% [4]. 65-70% of the total jaggery is made from sugar cane [5]. Different processing methods such as thermal treatment change the contents of the food components. Bioactive compounds are highly susceptible to production methods including conditions during processing [5]. The bioactivity of natural sugar depends on the phytochemicals that it contains and may vary according to its manufacturing method [6]. Phytochemicals are chemicals naturally present in plants which are natural compounds obtained from plants that occur majorly in the foodstuff plants having clinical importance. Unlike pharmaceutical chemicals, phytochemicals do not have any side effects [7]. There is also a simultaneous usage of methods for estimating the effectiveness of such substances as antioxidants [8]. Phytochemicals can be called or considered as "Human - friendly medicines"[9]. The least processed sugar jaggery retains a lot of the phytochemicals present in sugarcane juice and has several health advantages [10]. There is always a possibility of the phytochemicals being degraded or modified during the jaggery manufacturing process. Hence, a change in the bioactive potential may be expected compared to raw sugarcane juice and vacuum pan jaggery. Currently, negligible numbers of jaggery units are employing the vacuum pan method for the manufacturing of jaggery. In an open pan jaggery process, the phytochemicals present in the sugarcane juice are subjected to uncontrolled variable temperatures often very high. Adopting alternative technologies under the vacuum process the temperature in this process is controlled and does not exceed 70°C. Hence, the chances of photochemical degradation or modification are limited. The chemical components are subjected to oxidation in an open pan

process. Due to high-temperature charring occurs particularly at the bottom of the pan [11]. In an artisanal sugarcane process produced often by a process of open pan heating [12]. As mentioned in the literature, Jaggery retains phenolics and other phytochemicals with intense biological activities, including antioxidants, cytoprotective, and anthelmintic [13, 14]. The constituents found in sugarcane varieties have been mentioned to be anti-inflammatory, antioxidants and anti-inflammatory [7, 8]. A wide variety of biological extract activity in the sugar cane and its derived by products caused by the presence of various phenolic compounds [15]. The presence of antioxidant properties in jaggery is part of the widespread interest in antioxidant phenols that seek to exploit their potential health benefits and effect through food [16]. The range of nutritional components, bioactive of non-centrifugal cane sugar is influenced by the conditions of industrial processing [17]. Although there are several literature and reviews [18, 3], there is a gap in the existing of phytochemicals and biological studies of various types of sugarcane products in the handling of sugar cane derivatives such as, molasses, filter mud and bagasse. Further study is required to explain the variations in non-centrifugal cane sugar bioactive compounds during the heating phase [19]. The lacunae in the scientific literature related to phytochemical constituents of jaggery lead us to come out with the current investigation. As we have investigated the phytochemical profiles (phenolics, flavonoids, tannins, saponin, alkaloids, terpenoids) content, of jaggery aqueous extracts forms of lyophilized sugarcane products which were sugarcane juice, vacuum pan jaggery, and open pan jaggery, the antioxidant potential of the jaggery extracts was also investigated by three separate assays. In conclusion, the purpose of this research was to examine the biological properties of antibacterial action, including antioxidant. Such research will provide us with a deeper understanding of improve-ment in their possible beneficial impact on human health.

2. MATERIAL AND METHODS

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis 3-ethylbenzothiazoline-6-sµlfonic acid (ABTS), Bovine serum albumin, Tannic acid, Catechin, Gallic acid, Securigerasecuridaca, Mueller-Hinton agar, Nutrient agar, Nutrient Broth, etc; was purchased from Himedia Laboratories, Mumbai, India, and other chemicals like Methanol, Ethanol, Butanol, Ammonium hydroxide, Diethyl ether Ferric chloride, Potassium ferricyanide, Calcium hydroxide, diammonium salt, Folin-Ciocalteu reagent, Potassium acetate, Wagner's reagent, Sodium hydroxide, Sodium chloride, Hydrochloric acid, Ninhydrin, Glacial acetic acid, Chloroform, Sulphur acid, Molisch's reagent, Aluminum chloride, Hydrogen peroxide, Methylene blue, Potassium phosphate (monobasic and dibasic), Sodium bicarbonate, Bradford reagent, 2-thiobarbituric acid and Trichloroacetic acid used in the study were procured from a local chemical supplier, Bangalore.

2.2. Collection of Raw Sugarcane Sample

Collection of the sugar cane (VCF-0517) [20] was done from the local agricultural research station V.C. farm, Mandya, Mysore, Karnataka, India.

2.3. Sugarcane Juice Extraction

The outer skin of sugarcane was washed, cleaned and the sample weight was taken, then the Sugarcane juice was extracted using a modern electrical three-roller extractor. The sugarcane juice sample was used for the preparation of lyophilization sugar cane juice, vacuum pan jaggery, and open pan jaggery.

2.4. Sample Preparation

2.4.1. Preparation of Lyophilized Sugar Cane Juice, Vacuum Pan, and Open Pan Jaggery

The extracted juice was filtered and the pH was adjusted to being 7 (just for vacuum pan and open pan jaggery), for the open pan jaggery sample the juice was then boiled at 115°C, and concentrated until becoming thick syrup, followed cooling with stirring till solid jaggery is formed. The juice of vacuum pan Jaggery sample was evaporated using a vacuum evaporator at 65°C, vacuum pressure was set at 120 psi., and concentrated until getting thick syrup, then the sample produced was lyophilized and stored in a deep freezer until further use. To prepare the Sugar cane juice sample, sugarcane juice was filtered, lyophilized, and stored at -20°C till further use, then the prepared vacuum pan jaggery and open pan jaggery samples were lyophilized and stored (figs. 1-3). For the same, immediately before sample preparation, the row sugarcane is collected and juice extracted.

2.4.2. Extraction of Lyophilized Samples

A hundred grams of each sample was mixed in 500 ml deionized water, then shaken at 500 rpm for 24 h. The extract has been purified with grade 1 sterile Whatman paper filter and placed in a cooler before further use [21]. To concentrate samples, the crude extract was

evaporated to one-fourth of the initial sample volume using distillation. The concentrated samples were stored under refrigeration for further analysis.

Fig.1: Lyophilized open pan jaggery **Fig. 2: Lyophilized vacuum pan jaggery** **Fig. 3: Lyophilized sugarcane juice**

2.5. Physiochemical characteristics of samples

2.5.1. pH

The determination of pH was done using Comsys Digital pH meter. The samples of sugarcane juice, vacuum pan jaggery, and open pan jaggery after lyophilization were dissolved in sterile distilled water and the pH was noted down.

2.5.2. Water Activity

Capacitance or electric hygrometers were the instruments used to assess water activity. As measured by a sensor calibrated to defined saturated salt standards, the resulting change in capacity is almost equal to the activity of the water. The signal is provided by the sensor relative to the ERH and hence to the activity of the water (as ERH/100). The water activity was determined by putting the sample in a closed chamber and by measuring the relative humidity of the headspace, the liquid-phase water is mixed with the vapor-phase water. The ratio of the water vapor pressure of the sample to the water vapor pressure of the water vapor, divided by 100, is given as the water activity.

2.5.3. Moisture Content

Moisture analysis was done by the oven drying method [22] as per using CHEMI Digital Hot Air Oven. The initial moisture content of the lyophilized Sugarcane juice, vacuum pan jaggery, and open pan jaggery was determined. The initial weight of 2g of the samples was individually recorded and placed in a clean dry petri dish and dried at 105±3°C in a hot air oven for 24 hours, the sample was weighed again to check the weight after 24 hours till a constant weight was observed for each sample. The moisture content tests were carried out in triplicate and weighing was done to the nearest ±1 mg. The moisture content of the sample was calculated using the following equation.

Content of Moisture = [(Initial weight- Dry weight of the oven) / Dry weight of the oven] * 100

2.6. Phytochemical analysis

2.6.1. Quantitative

Triplicates of the samples were performed for Quantitative phytochemical as follows:

2.6.1.1. Determination of total phenol content (TPC) [23]

2.5 ml of F.C reagent was applied to 500 µl of the sample concentrate and incubated for 5 min at room temperature, added 2 ml of 7.5 percent sodium carbonate solution, combined 25 ml of the sample with filtered water and incubated for 2 h in the dark. The absorption was estimated at 765 nm. Gallic acid was used as standard. The product of phenol was expressed in mg GAE/ml (gallic acid equivalent).

2.6.1.2. Determination of Flavonoids Content (TFC) [23]

Five (5) ml of 2% $AlCl_3$ prepared in methanol was combined with 5ml extract solution. The absorption at 415 nm against a blank was taken after 10 mins. As an

extract of 5 ml combined with methanol without $AlCl_3$. As the standard, Catechin has been used as (mg CE/ml.).

2.6.1.3. Total Tannin content (TTC) determination

The tannin solution was estimated using the procedure [24], 1 ml of the sample extract was blended with 7.5 ml of purified water and 0.5 ml of folin phenol reagent and 1 ml of sodium carbonate (35%) was applied. The tannic acid has been used as a standard. The findings of tannins are expressed as tannic acid in mg/ml of extract.

2.6.1.4. Determination of Saponin content [25]

For 20 ml of 20 percent ethanol, 1ml of each sample was dispersed. The suspension was heated for 4 hours with constant stirring at around 55°C over a hot water bath. For an additional 20 ml of 20 percent ethanol, the mixture was purified and the residual re-extracted. In a hot water bath at around 90°C, the mixed extracts were reduced to 40ml. In a 250 ml separating funnel, the concentrate was moved, and 2 ml of Diethyl ether was applied and vigorously shaken. The aqueous layer was collected and there was a repeat of the purification process. 6ml of n-butanol was added to this and the combined extracts of n-butanol were washed twice with 1ml of 5% aqueous sodium chloride. In a water bath, the remaining mixture was heated. The samples were dried in the oven at a steady weight after evaporation.

Saponin = Weight of Saponin/ml of the sample taken

2.6.1.5. Total Alkaloid content (TAC) determination

In 100 µl of the sample, 40ml of 10 percent acetic acid in ethanol was applied, covered, and allowed to stand for 4 hours. Until it drops to $1/4^{th}$ of its original volume, the filtrate was enabled to concentrate on a water bath. When the precipitation was complete, condensed ammonium hydroxide was applied dropwise to the extract. It was allowed to settle the whole solution, washed the accumulated precipitate with dilute ammonium hydroxide, purified, dried the residue and weighed.

Alkaloids = (weight of Alkaloids)/(Volume of the sample)

2.6.1.6. Terpenoids content (TC) determination [26]

1.5 ml of chloroform was added in 200 µl of sample and allowed to settle for 3 min. Afterwards, 100 µl of conc. sulfuric acid was added and placed on ice to prevent heating (not more than 15 min). Placed in dark for incubation for 1.5-2 h (For standard solution not more than 5min incubation). After incubation supernatant was discarded. 1.5 ml of methanol was added and OD was read at 538 nm. The calculation of total terpenoid content was done by the Linalool equivalent.

2.6.1.7. Protein content (PC) determination [27]

Different volumes of the sample extract were mixed with 3ml of Bradford's reagent and incubate in dark for 5mins. The absorbance was measured at 595nm. Bovine serum albumin (BSA) was used as standard.

2.7. Antioxidant assay

The Antioxidant assay of SJ, VJ, and OJ aqueous extract was estimated by three in-vitro assays, ABTS, DPPH, and reducing power assay. All assays were conducted in triplicate.

2.7.1. ABTS radical scavenging assay

Following the technique described [28], antioxidant potential was evaluated in terms of $ABTS^+$ radical scavenging activity. In short, by reacting with 7mM ABTS stock solution and 2.45mM potassium persulfate, $ABTS^+$ was obtained. The mixture was stored at room temperature for incubation in the dark for 12-16 h until application. The $ABTS^+$ solution was diluted with 5mM saline phosphate-buffered (pH 7.4) to absorb 0.70 ± 0.02 at 730nm. Afterwards 10 µl sample was applied to 4ml of diluted $ABTS^+$ solution, the absorbance was assessed for 30 minutes. Both specimens have been examined in triplicate. The samples' $ABTS^+$ radical scavenging operation was represented as

I %= (A control-A sample)/A control * 100

The absorption of the blank control (ABTS+ solution of test samples) is A control and the absorption of the test sample is A sample. The inhibition concentration (IC50) values for each sample were determined by a basic equation using Graphpad prism [29].

2.7.2. DPPH Method (1,1 diphenyl 2, picryl hydrazyl) [11]

This is the most commonly documented approach for the screening of such plant drugs for anti-oxidant effect. The procedure of DPPH evaluation is based on the reduction by a free radical scavenger of the colored free radical DPPH methanol solution. The operation is representted as a concentration that is efficient. In five test tubes, crude extracts with varying concentrations were taken. Volume was made up to 1ml with

methanol. 3ml of DPPH was added to each tube and incubated for 30 mins in dark. Absorbance was measured at 517nm against a suitable blank. The blank was prepared for each with methanol (1ml) and DPPH (3ml) without the samples Likewise, the samples' DPPH radical scavenging operation was represented as

I %= (A control-A sample)/A control * 100

The inhibition concentration (IC50) calculation for DPPH assay was done for the samples using the same equation [29].

2.7.3. Reducing power assay *[30]*

The sample solvent extraction was done in different concentrations in each test tube and up to 1 ml of solvent make-up. To each test tube, 0.5ml of 0.2M phosphate buffer and 0.5ml of 1% potassium ferrocyanide were added. 0.5ml of 10 percent trichloroacetic acid was applied after incubating the mixture at 50°C for 20 minutes and then the mixture was centrifuged for 10 minutes at 3000rpm. 1 ml of supernatant was combined with 0.1 percent ferric chloride solution and 1 ml of sterile water and 0.2 ml. The absorbance was measured by a spectrophotometer at 700nm. IC_{50} measurements were conducted for the reduction of power assay and values were noted [29].

2.8. Antimicrobial Activity

Antimicrobial activity of the extracted sugarcane juice, open pan jaggery, and vacuum pan jaggery samples was done by Agar Well diffusion method [31] using Mµller Hinton Agar (MHA- Hi-Media) against 10 different clinical isolates *(Proteus vµlgaris*, *Staphylococcusaureus, Pseudomonas aeruginosa, Candida albicans, Bacillus subtilis, Klebsiella pneumoniae, Streptococcus mutans, Salmonella typhi, Escherichia coli, and Enterococcus faecalis*). Overnight fresh cultures of the pathogens in the nutrient broth were swabbed onto MHA plates and well punched using a 6mm sterile punch. 80µl of the sample was added to each well. The samples were labelled as per the extract, respectively. The experiment was conducted in triplicates and the plates were incubated at 37°C for 24 hours and the zone of inhibition was recorded in mm and the mean values of the triplicates were determined and SEM was calculated.

2.9. Statistical analysis

The statistical analysis was done for each parameter, and the means values compared as per Duncan's least significant difference test at (P≤0.05). All statistical analyses were carried out using the software package SPSS (version 22.0).

3. RESULTS AND DISCUSSION

3.1. Physico-chemical characterization

3.1.1. Sugarcane juice pH

Sugarcane juice pH was assessed before the samples were prepared and after lyophilization. The values of the pH SJ sample were observed to be marginally reduced from 5.2 to 5.15, while values for VJ and OJ increased significantly to 6.36 and 6.13, respectively. Due to the addition of calcium hydroxide to modify the pH for jaggery preparation, the drop in acidity triggered a concomitant rise in the pH value, suggesting that the pH value influenced by the jaggery process variance as the VJ and OJ samples were still considered to be a low acid product as their pH value was higher than 6, which agreed with the findings stated by [32]. Lyophilized Jaggery samples showed high pH as recommended by the Ecuadorian technical standard [33] for panela with a minimum pH of 5.90, and the lower acidity of jaggery could be attributed to insufficient addition of lime during juice clarification, [34]. Upon heat treatment on sugarcane juice, pH reduction was reported, these findings illustrate that non-thermal treatments could have milder effects on the physicochemical properties of the products because they are less invasive than heating [35].

3.1.2. Water activity (aw)

Water activity (aw) evaluates the free/active water present in jaggery relevant to microbial growth, and it's one of the main factors for the spoiling of Jaggery or sugarcane products through extended storage. It is observed from the results that obtained increased significantly for values of VJ and OJ 0.4742 and 0.4853 aw respectively compared with SJ the least water activity of 0.3669 aw. The results indicate that the water activity is low enough to discourage the growth and spoilage by microorganisms as three samples showed a lower range of water activity than the optimum range for the growth of microorganisms [32].

3.1.3. Moisture Analysis

Water content determines the shelf life of any food, the higher moisture or the lower the shelf life or is vulnerable to spoilage due to contamination by micro-organisms. The Indian Standards Bureau, BIS 1990, sets out 5-7% of fresh jaggery moisture [36]. For the present study, the values of moisture content were significantly

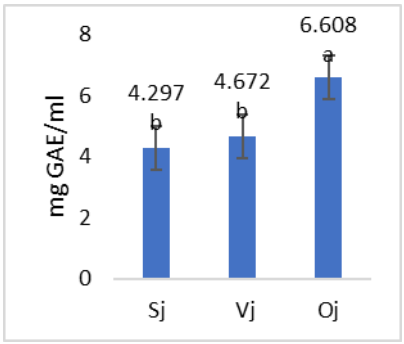
different as observed on values for the different samples for VJ, SJ, and OJ which were 5.93, 4.72 and 3.52% respectively. The processing of the jaggery and crystallization thereafter lower the moisture content and hence improves the shelf life. After lyophilization, it was observed that the sugarcane juice and vacuum pan jaggery was highly hygroscopic and absorbed moisture meeting atmospheric air and hence the increase in the moisture content. A research has shown that hygroscopicity not only depends on the temperature, but also on the water activity, crystal size and other food compounds [37].

3.2. Phytochemical analysis

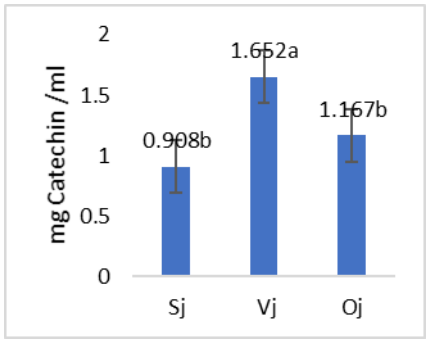
3.2.1. Phenol content

The mean values of phenol content of SJ, VJ and OJ are given in Fig. 4-A. There were significantly higher OJ which was 6.608 mg GAE/ml compared with VJ and SJ which were 4.672 and 4.29 mg GAE/ml respectively.

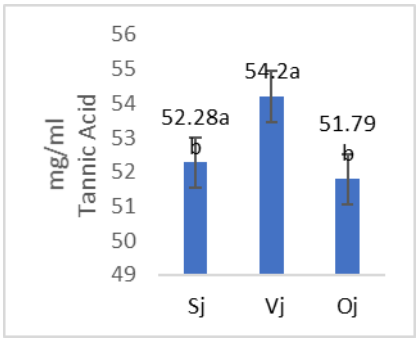
Regarding the processing effect on total phenolic content, the results are following the previous finding reported [38, 39]. As suggests an apparent increase in the value of phenolic compounds in medium products and by-products, provided that the sugarcane juice is 2.67±0.04 mg GAE/g TPC. In molasses backed by jaggery, higher phenolic contents were found, phenolic content in molasses, jaggery, 3751 lg GAE/g, 3285 lg GAE/g, respectively [1]. The investigators reported that increased phenolic compounds in downstream manufacturing by-products were correlated with the release of aglycones from phenolic glycosides found in sugarcane juices, resulting in higher concentrations in molasses and vinasse of these free phenolic structures [4, 17]. Scientists [3, 40] reported that some of the phenolic compounds contained in molasses could be obtained due to the degradation of hydroxycinnamic acid derivatives existing in sugarcane juices.



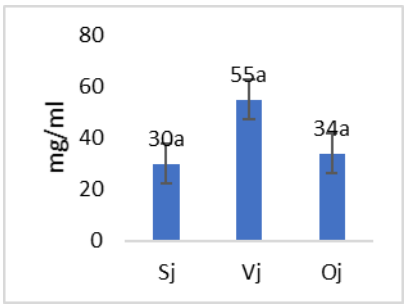
A. Phenol Content



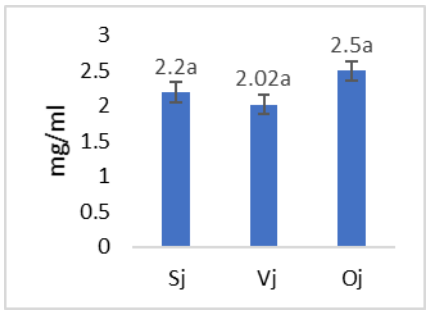
B. Flavonoids Content



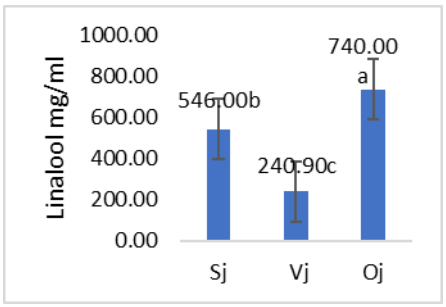
C.Tannin Content



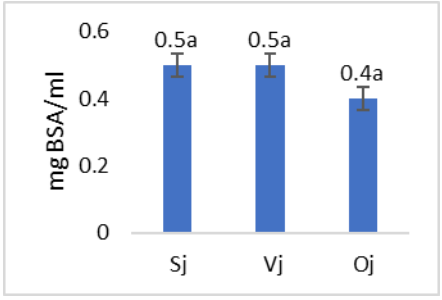
D. Saponin Content



E. Alkaloid Content



F. Terpenoids Content



G. Protein Content

Different letters in the same figure indicate significant differences among means ($p < 0.05$)

Fig. 4: Quantitative Phytochemical analysis of SJ, VJ and OJ

3.2.2. Flavonoids Content

Flavonoids and tannins are secondary metabolites that have different biological properties and are widely distributed in the plant kingdom [41]. The results of the present study Fig. 4-B indicated high significance in the mean value of total flavonoid content between the different samples for VJ, OJ and SJ which were 1.652, 1.167 and 0.908 mg CE/ml respectively. Previous studies reported that the sugarcane is rich in flavonoid compounds, the TFCs were comparable (259 mg QE/ 100 g extracts and 297 mg QE/100 g,) [42, 43]. These results are consistent with [18] that revealed a flavonoid content in the range of 170 mg of flavonoid/100 g of fresh plant material in sugarcane. The high content of flavonoids in sugar cane juice and molasses provides possible alternatives to other natural resources [2], such as apples (98-143 mg flavonoid/100 g of fresh plant material) [40, 44, 18]. Flavonoids with the most potent antioxidant ability are the most abundant type of dietary polyphenols due to their differentiated functional groups [45]. In this context, sugarcane juice can be used as a source of antioxidants. The phenolic compound mainly flavon-O and C-glycosides have been found to play an important role as antioxidants [46].

3.2.3. Tannin content

The mean values of tannin content increased significantly which were 54.20, 52.28 and 51.79 mg/ml tannic acid for VJ, SJ and OJ respectively as presented in Fig. 4-C. [47] reported that the tannin content may be bound to proteins nearby (pH 3.5-7.5) to form tannin–protein structures separating at a pH of 3.5.

3.2.4. Saponin content:

Saponin content values as shown in Fig. 4-D.were not significantly different between different samples.

3.2.5. Total alkaloid

The mean values of total alkaloid content in the extracted samples were not significantly different between different samples as shown in Fig. 4-E.

3.2.6. Terpenoid content:

The results showed in Fig. 4-F a highly significant increase in the content of terpenoid for OJ, SJ and VJ which were 740.00, 546.00 and 240.90 Linalool equivalent mg/ml respectively.

3.2.7. Protein content

The mean values of protein content were not significantly different between the samples as presented in Fig. 4-G. As reported by Hagerman and Butler [48], (BSA) was used as a protein for this analysis. Many studies have shown that the presence of several amino acids in raw sugarcane juice and molasses during the boiling step of the manufacture of molasses may derive from protein hydrolysis [28, 49].

3.3. Antioxidant assay

3.3.1. ABTS (2,2'-azinobis (3-ethylbenzothiazoline -6-sµlfonic acid) radical scavenging assay

The $ABTS^{+}$ radical scavenging activity of the samples was calculated by measuring the absorbance value of the blank control and absorbance value of the sample, which then was expressed as a percentage value. The samples extracted from SJ, VJ, and OJ showed a very high level of scavenging activity, and the ability to scavenge increased with an increase in the concentration of the samples. The SJ exhibited scavenging activity ability to 94.84% similarly OJ was relatively high with 92.25% and VJ 85.46.the IC50 values for different antioxidant assays of SJ, VJ and OJ are given in Table 1. The highest IC_{50} showed highly significant for SJ 0.632, and for VJ was higher (insignificantly) than OJ sample 2.331 and 2.441 respectively..

The antioxidant potential of plant extracts is due to the inclusion of polyphenols in the extracts and their ability to govern as hydrogen atom or electron donors and to catch free radicals in the solution. Other experiments have shown that polyphenolic compounds, and in particular flavonoids, have been proposed as medicinal options for the treatment of many pathologies [50]. Total 35 phenolic compounds (most of which belong to the flavonoid group) have been tested for inhibiting free radicals [49, 51, 52].

Table 1: IC_{50} values for different antioxidant assays with SJ, VJ and OJ

Sample	IC50 values for ABTS assay	IC50 values for DPPH assay
SJ	0.632±0.10 b	0.073±0.01 b
VJ	2.331±1.00 a	0.373±0.10 b
OJ	2.441±1.00 a	2.810±1.00 a

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

Several flavonoids of Saccharum species have been documented in a previous literature review [53]. More recently, commercially grown sugarcane in Brazil (*Saccharum officinarum* L.) is under systematic study due

to the possible function of this plant as a nutritional source of flavonoids with antioxidant properties [46, 54]. The association between total polyphenols and antioxidant activity is usually strong but depends on the characteristics of the samples and the influence of other compounds on antioxidant activity [55]. Absolute polyphenols and antioxidant function were not substantially associated in another report [40].

3.3.2. DPPH Assay (1,1-Diphenyl- 2- picryl hydrazyl)

The DPPH antioxidant assay is based on the reduction of the colored free radical DPPH methanol solution by a free radical scavenger. The activity is expressed as an effective concentration. Similar to the ABTS radical scavenging assay result, the sugarcane juice and the differently processed jaggery samples showed concentration-dependent free radical scavenging activity *i.e.* increase with a higher concentration of the samples. The results suggest VJ having higher scavenging activity with a 79.65% than OJ 71.42% where was 89.69% for SJ that indicates the unprocessed sugarcane juice having higher antioxidant activity due to intact phytochemicals compared to the processed jaggery samples. The IC_{50} values for different antioxidant assays of SJ, VJ and as showed in Table 1. The highest IC_{50} was observed for 0.073 significantly, compared with VJ and OJ which were 0.373 and 2.810 respectively.

The Co 419 jaggery variety has a lower association between phenolic content and radical scavenging ability [56]. A statistically negative association with phenolic content ($p<0.01$) with $r=-0.872$ was demonstrated by IC_{50} values of DPPH scavenging ability of the bound fractions, indicating that DPPH scavenging capacity was positively associated with bound phenolics. In deciding sweet corn types, however, free phenolic content did not demonstrate a meaningful association with the scavenging potential of DPPH. The CAA values expressed as per 100 g sweet corn fresh weight were slightly positive associated with the phenolic contents with free fraction $r = 0.806$ ($p < 0.05$) and inbound fraction $r = 0.934$ ($p < 0.01$) [57]. Brown sugar aqueous solutions demonstrated poor free radical scavenging potential to assay DPPH [40].

3.3.3. Reducing power assay

In the case of reducing power antioxidant assayit was concluded that the higher levels of antioxidant activity might be due to certain phytochemical-like-flavonoids. Reducing effects are usually related to the presence of reductions, which have been shown to exert antioxidant activity by splitting the free radical chain by contributing an H^+ atom. Reductions also react to certain peroxide precursors, which prevent the formation of peroxide. 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 higher activity with VJ, SJ and OJ which were 0.150, 0.147, and 0.108% respectively, activity indicating that vacuum pan jaggery had a higher inhibitory activity compared to open pan jaggery. In sugarcane juice, the overall phenolic and flavonoid content was found to be directly proportional to their antioxidant effects [58]. Because of their distinct functional classes, flavonoids with the most effective antioxidant potential are the most abundant form of dietary polyphenols [45]. Jaggery group previously stated that the literature indicated *E. cardamomum* antioxidant activity. There was no link between the cardamom and its phenolic compounds. In their investigation, however, *E. cardamomum* addition greatly improved both phenolic content and antioxidant potential [56].

3.4. Antimicrobial Activity

In the antimicrobial testing of the samples, all the organisms showed significant differences between the samples as presented in Table 2. In the zone of inhibition for Aqueous extract of Sugarcane Juice (SJ), Vacuum Pan Jaggery (VJ) and Open Pan Jaggery (OJ) except *Salmonella typhi. Bacillus subtilis and Pseudomonas aeruginosa* too did not show a zone of inhibition for OJ.

In the case of vacuum pan jaggery extract (SJ) except for *E Coli* and *Staphylococcus aureus* all the organisms were found to be resistant. *Staphylococcus aureus* was the most sensitive organism and showed the maximum zone of inhibition with 31mm followed by *E Coli* and *Pseudomonasaeruginosa* with 29mm and 24mm, while *Salmonella typhi* was the most resistant for all the extracts. The result suggests aqueous samples have very high efficiency as an antimicrobial agent due to the presence of very high sugar concentration and also certain phytochemicals present in the sugarcane extract. The antibacterial activity may be attributed to the polyphenols and antioxi-dant properties of the jaggery [19, 59, 60]. In previous experiments, antibacterial activity was found to be closely related to phenolic and flavonoid concentration and thus to the antioxidant potential of the jaggery.

Table 2: Antimicrobial activity of SJ, VJ and OJ against clinical pathogens

S	Conc. 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/ml	30.00±1.00^{a}	16.66±2.08^{a}	16.33±1.53^{a}	22.67±1.53^{a}	14.00±1.00^{a}	8.00±1.00^{a}	20.33±1.15^{a}	18.67±0.57^{a}	21.67±3.2^{a}	22.33±3.05^{a}
VJ	0.933g/ml	28.67±1.53^{a}	6.33±5.68^{b}	8 .00±0^{c}	9.00±1.00^{c}	8.00±1.00^{c}	9.33±1.53^{a}	0±0^{c}	3.67±6.35^{b}	21.67±1.53^{a}	0±0^{b}
OJ	0.954g/ml	24.00±1.73^{b}	13.00±1.00^{ab}	10.67±0.58^{b}	13.33±1.53^{b}	11.67±0.58^{b}	0±0^{b}	9.33±1.52^{b}	8.33±0.58^{b}	14.00±2.64^{b}	22.00±6.55^{a}

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

4. CONCLUSION

The obtained results indicate that the samples extracted from sugarcane juice, vacuum pan jaggery and open pan jaggery contained alkaloids, flavonoids, saponins, tannins, phenols, proteins, and, terpenoids. The results showed that the samples extracted from sugarcane juice, vacuum pan and open pan jaggery contained a high level of tannin, flavonoid, terpenoid, and phenols. The total content of proteins, saponin, and alkaloid was comparatively low. The pH of the sugarcane juice decreased on lyophilization to the acidic range but the jaggery products were slightly towards the neutral range. The water activity indicated decreased available water for the growth and proliferation of micro-organisms thus increasing the shelf life and similarly low water content in the lyophilized samples through sugarcane juice and vacuum pan jaggery were highly hygroscopic after the process and had the tendency to absorb moisture, open pan jaggery was stable in its moisture content. On the other hand, the in-vitro antioxidant activity assay determined using ABTS radical scavenging assay, DPPH, reducing power assay indicated the presence and expression of phenolic and flavonoid content. The antimicrobial activity showed that all three samples especially SJ to have potent antimicrobial properties especially against *Staphylococcus aureus* and *Escherichia coli*. Hence Sugarcane juice and its byproducts Jaggery can be used not only as a refreshing drink as an energy booster but also with a lot of health benefits. The variation in the phytochemicals in the sugarcane juice, vacuum pan jaggery, and open pan jaggery may be due to the natural and the processing procedures followed with exposure of the juice to heat which can vary the chemical composition of the sample. But the presence of all the phyto-constituents indicates aqueous extract as a universal solvent capable of extracting all the chemicals and the compounds miscible in the same.

5. ACKNOWLEDGMENTS

The authors thank Dr. Swamy Gowda, Sugarcane Breeder, Zonal Agricultural Research Station, V.C. Farm, Mandya, Karnataka, India for providing sugar-canes variety for our study. Muneer S.H Ahmed wish to thank the Department of Sugar Technology, Sir. M. Visvesvaraya, Post Graduate Centre, University of Mysore, Mandya 571402, Karnataka, India, for providing infrastructure for the research work.

Conflicts of interest

We declare that there are no conflicts of interest.

6. REFERENCES

1. Food and Agriculture Organization of the United Nations (FAO), Sugarcane production quantities by country. Accessed 16 Feb 2016.
2. Rao JPVK, Das M, Das SK. *Indian Journal of Traditional Knowledge*, 2007; **6(1):**95-102.
3. Anon. *Cooperative Sugar,* 2005; **36(6)**:516.
4. Kumar K. Oscillations in Jaggery and Khandsari industry, Proceedings of the natural seminar on status, problems and prospects of jaggery and khandsari industry, 1999; pp 87.
5. Iqbal M, Qamar MA, Bokhari TH, Abbas M, Hussain F, Masood N, et al. *Information Processing in Agriculture*, 2017; **4(1):**83-89.
6. Doughari JH, Elmahmood AM, Manzara S. *African Journal of Microbiology Research*, 2007; 037-041.
7. Maurício Duarte-Almeida J, Novoa AV, Linares AF, Lajolo FM, Inés Genovese M. *Plant Foods for Human Nutrition*, 2006; **61(4)**:187-192.
8. Takara K, Matsui D, Wada K, Ichiba T, NakasoneY. *Biosci Biotechnol Biochem,* 2002; **66(1)**:29-35.
9. Sahira Banu K, Cathrine L. *International Journal of Advanced Research in Chemical Science*, 2015; **2(4)**:25-32.
10. Walter Jaffe R. *Sugar Tech*., 2012; **14**:87-94.
11. Ejo Grantham. *Proc S AfrSug Technol Ass,* 2001; 75.
12. Kamble KD. *Indian Journal of Traditional Knowledge*, 2003; **2(2)**:137-147.
13. Harish Nayaka, MA, Sathisha UV, Manohar MP, Chandrashekar KB, Shylaja MD. *Food Chemistry,* 2009; **115**:113-118

14. Prasad P, Kumar P, Satyavathi, K, Prabhakar MC. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 2010*;* **1**:510-514.
15. Sreeramµlu D, Reddy CVK, Anitha C, Balakrishna N, Raghunath M. *Oxidative medicine and cellµlar longevity*, 2013; 1-12.
16. Scalbert A, Johnson IT, Saltmarsh M. *Am J Clin Nutr*; 2005; **81**:2158-2178.
17. Jaffé W. *J. Food Compos. Anal.,* 2015*;* **43**:194-202.
18. Colombo R, Lancuas F, Yariwake M. *J. Chrom. A.*, 2006; **1103**:118-124.
19. Velásquez F, Espitia J, Mendieta O, Escobar S, Rodríguez J. *Journal of Food Engineering,* 2019; **255***:* 32-40.
20. Swamygowda SN,Nagaraja TE, Keshavaiah KV. *Journal of Agricµltural Sciences*, 2017; **51(4)**:803-807.
21. Zhang QW, Lin LG, Ye WC. *Chin Med.*, 2018; 13-20.
22. Reeb J, Milota M. Moisture Content by the Oven-dry Method for Industrial testing. Oregon State University, Corvallis, OR. WDKA, 1999.
23. Ramamoorthy PK, Bono A. *Journal of Engineering Science and Technology*, 2007; **2**:70-80.
24. Hasan AHM. *Food Measure,* 2015; **9**:454-462.
25. ObadoniBO, Ochuko PO. *Global Journal of Pure and Applied Science*, 2001; **8**:203-208.
26. Suica-Bunghez, IR, Teodorescu S, Dµlama ID, Voinea OC, Imionescu S, Ion RM. *IOP Conf. Ser. Mater. Sci. Eng.*, 2016; **133(1)**.
27. SundarAR, Velazhahan R, Nagarathinam S, Vidhyasekaran P. *Biol Plant*. 2008; **52**:31-32.
28. Delgado-Andrade C, Morales FJ. *Journal of Agricµlture and Food Chemistry*, 2005; **53**:1403-1407.
29. ChenZ, Bertin R, Froldi G. *Food Chemistry*, 2013; **138(1)**:414-420.
30. Jayanthi P, Lalitha P. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011; **3**:126-128.
31. Nair R, Kalariya T, Chanda S. *Turk J Biol,* 2005; **29**:41-47.
32. Guerra MJ, Mujica MV, *CienciaeTecnologia de Alimentos Campinas,* 2010; **30**:250-257.
33. Ecuadorian Technical Standard NTE INEN 2 332. Panela GranµladaRequisitos, 2002; p.2 Quito-Ecuador
34. Jittanit W, Wiriyaputtipong S, Charoenpornworanam H, Songsermpong S. *Chiang Mai Journal of Science*, 2011; **38(1)**:116-125.
35. Jiménez-Sánchez C, Lozano-Sánchez J, Segura Carretero A, Fernández-Gutiérrez A. *Critical Reviews in Food Science and Nutrition*, 2017; **57(3)**:637-652.
36. Anonymous. Bureau of Indian Standards, IS 12923,(Clause 4.3 and 6.1), New Delhi, India. 1990.
37. Mathlouthi M, Rogé B. *Food Chemistry*, 2003; **82(1):**61-71.
38. Awadallah RM, Sherif MK, Mohamed AE, Grass F. *J RadioanalytNucl Chem.*, 1985; **92**:7-25.
39. Molina-Cortés A, Sánchez-Motta T, Tobar-Tosse F, Quimbaya M. *Valorization*, 2020; **11**:3453-3463.
40. Payet B, Shum Cheong Sing A, Smadja J. *J. Agric. Food Chem.*, 2005; **53**:10074- 10079.
41. Naczk M, Pegg, RB, Amarowicz R. *Food Chem.*, 2011; **124**:1507-1513.
42. Godshall MA, Vercellotti JR, Triche R. *Int. Sugar J.,* 2002; **104**:228-233.
43. MabryTJ, Liu YL, Pearce J, Dellamonica G, Chopin J, Markham KR, et al. *J. Nat. Prod.*, 1984; **47**:127-130.
44. Eberhardt MV, Lee CY, Liu RH. *Nature*, 2000; **405**:903-904.
45. Bravo L. *Nutrition Reviews*, 1998; **56:**317-333.
46. Ferreira RQ, de Lira, TO, Zeraik ML, Yariwake JH, *J. Braz. Chem. Soc. LC/UV/MS and Post-Column Derivatization*, 2009; **20(9):**1574-1579.
47. Jones WT, Mangan JL. *Journal of the Science of Food and Agriculture*, 1979; **28(2)**:126-136.
48. Hagerman AE, Butler L. *J. Agric. Food Chem.,* 1978; **26**:809-812.
49. Aquino FW, Boso LM, Cardoso DR, Franco DW. *Food Chem.*, 2008; **108**:784-793.
50. Graf BA, Milbur PE, Blumberg JB. *J Med Food,* 2005*;* **8(3)**:281-290.
51. LeeSK, Mbwambo ZH, Chung H, Luyengi L, Gamez EJ, Mehta RG, et al. *Comb Chem High Throughput Screen,* 1998; **1(1)**:35-46.
52. Cholbi MR, Paya M, AlcarazMJ. *Experientia,* 1991; **47(2)**:195-199.
53. Smith P, Paton NH. *Sugar Technol. Rev.,* 1985; **12**:117.
54. Vila FC, Colombo R, de Lira TO, Yariwake JH, *J. Braz. Chem. Soc.* 19, 903 and references cited herein, 2008.
55. Moure A, CruzJM, Franco D, Dominguez JM, Sineiro J, Dominguez H, et al. *Food Chem.*, 2001; **72**:145-171.
56. Chandrakanth, V, Harish Nayaka MA, Shivalingaiah, S, Chikkappaiah L. *Sugar Tech*, 2019; **21:**388-397.

57. ZhangR, Huang L, Deng Y, Chi J, Zhang Y, Wei Z, Zhang M. 2017; **20(12):**3043-3055.
58. Kadam US, Ghosh SB, Strayo De, Supra-sanna P, Devasagayam TPA, Bapat VA. *Food Chem*., 2008; **106**:1154-1160.
59. Shan B, Cai YZ, Sun M, Corke H. *J Agric Food Chem*., 2005; **53**:7749-7759.
60. Harish Nayaka MA, Chikkappaihi L, Venkatesh KS, Gunashree BS, Sudharshan S. *Asian J Pharm Clin Res.*, 2018; **11(11):**294-299.