

## EVALUATION OF BIOACTIVITY OF JAGGERY PREPARED USING PLANT MUCILAGE AS CLARIFICANT

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### ABSTRACT

**Objective:** The aim of the study was to prepare the jaggery using plant mucilage as clarificants and to evaluate the bioactivity of jaggery by determining total phenols, total flavonoids, reducing power, antibacterial activity and antihelmintic activity.

**Methods:** Jaggery was prepared from sugarcane variety Co-80632 using five plant mucilage as clarificants, namely *Aloe vera*, flax seeds, fenugreek, purslane, and malabar spinach at three different concentrations, i.e., 0.1%, 0.2% and 0.4% of raw sugarcane juice. The characteristic bioactivities such as total phenol content by Folin-Ciocalteu method, flavonoids content by spectrophotometer method, reducing power assay by measuring the absorbance at 700 nm, antibacterial activity by well-diffusion method and antihelmintic activity by *in vitro* were determined.

**Results:** The highest total phenolic content was observed in jaggery prepared using plant mucilage clarificant of *A. vera* (JAV4 [3.68±0.02]) and Fenugreek seeds (JFG4 [3.61±0.02]) at 0.4% followed by them at 0.2% and 0.1% concentration, respectively. Similarly, a significant amount of total flavonoids content was elevated only in 0.4% plant mucilage clarificants of *Aloe vera*-treated experimental group jaggery (0.73±0.01) compared to control (0.61±0.02). Further, the antioxidant activity of jaggery was evaluated by reducing power. The increased absorbance at 700 nm indicated the presence of reducing power. At 10 mg/ml concentration, an absorbance unit of 1.93 and 1.80 was observed for jaggery prepared using 0.4% concentration of *A. vera* and fenugreek seed mucilage clarificants. Antihelmintic activity of jaggery showed dosage depended on the pattern where with the increase in the concentration of mucilage, the antihelmintic activity enhanced. *A. vera* mucilage showed maximum antihelmintic activity at 0.4% concentration with paralytic time (28.6 min) and death time (39.6 min).

**Conclusion:** Jaggery prepared using plants mucilaginous clarificants is the richest source of polyphenols such as phenolic acids and flavonoids, thereby contributed to the enhancement of bioactivity such as antioxidant, antibacterial and antihelmintic activity. Thus, jaggery prepared using plant mucilage as clarificants is a novel nutraceutical product which can supplement the nutrients along with various medicinal properties through antioxidant, antibacterial and antihelmintic activity.

**Keywords:** Jaggery, Plant mucilage, Clarificants, Total phenols, Antihelmintic activity.

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### INTRODUCTION

From times immemorial food habits of human beings included natural sweeteners and their importance recognized in Indian diets as well. Jaggery, the well-known sweetening agent, prepared by concentration of sugarcane juice is added to beverages and foods for increasing palatability. The practice of feeding jaggery to mothers and cows during the lactation period just after delivery is common in India [1]. Jaggery was reported to contain sucrose (65–85 %), reducing sugar (10–15%), proteins (4%), minerals (1%) and vitamins and phenolic compound (280–320 mg/100 g) [2]. Jaggery being a least processed sugar retains much of the phytochemicals that are present in sugarcane juice and exhibits various health benefits [3]. Indian Ayurveda medicine considers jaggery as a medicinal sugar and jaggery has been reported to possess protective effect against smoke-induced lung damage, arsenic-induced chromosomal aberrations, arteriosclerosis and cytoprotection against oxidative damage [4-7].

Sugarcane contains various phenolic compounds, and its extracts have displayed a wide range of biological activities including antioxidant, anti-inflammatory, antithrombosis, immune stimulation and anti-stress effects [8-11]. It is also reported that natural sweeteners such as honey, palm-jaggery, and cane jaggery to possess antihelmintic activity [12]. A study on antioxidant properties of fortified jaggery with *Neem* leaf has exhibited antioxidant activity [13]. The jaggery enriched with

ginger in different sugarcane varieties was reported to have elevated its antioxidants properties [14].

The interest in plant phenolics has considerably increased in recent years because of their possible role in the prevention of oxidative stress-induced diseases such as cardiovascular complications, diabetes, ulcers and cancer [15-18]. The search of antioxidants in jaggery is part of the extended interest in antioxidant phenolics driven by the quest of exploiting their putative health effect through food [19]. The bioactivity of these natural sugars can be anticipated, as they contain phytochemicals to a different extent depending on their manufacturing process.

The indiscriminate level of addition of chemical clarificants such as Hydrous, sodium carbonate, sodium bicarbonate, superphosphate and alum during jaggery production has led to downfall of jaggery market due to its poor keeping quality of jaggery during storage [20,21]. Due to health consciousness BIS has set up guidelines for the chemical clarificants and should be used within a safe limit. To overcome the effect of chemical clarificants residues in the jaggery, vegetable clarificants can be used as potential alternatives to replace chemical clarificants/synthetic clarificants. The wide uses of mucilaginous plants sources such as *Hibiscus ficulneus* (Deola), *Hibiscus esculentus* (Bhindi), *Cordia alliodora* (Sukalai), *Bombax malabaricum* (Semal bark), *Grewia asiatica* (Falsa), *Arachis hypogea* (Ground nut), *Recinus communis* (Castor seed),

*Manihot esculentum* (Tapioca) and *Glycine max* (Soybean) are reported in clarification of jaggery [20-23]. The mucilage from plant sources was already exploited in the pharmaceutical, cosmetic field and their therapeutical application [24].

In the present study, the mucilage extracted from *A. vera*, flax seeds, fenugreek seeds, purslane and malabar spinach was used as clarificants at three different concentrations, i.e., 0.1%, 0.2%, and 0.4% of raw sugarcane juice. The biological activity of jaggery prepared using plant mucilage clarificant was determined by evaluating total phenol content, total flavonoids content, reducing power, antibacterial activity and antihelmintic activity.

## METHODS

### Samples

The sugarcane variety Co-86032 of 10 months age was collected from Zonal Agricultural Research Station, V.C. Farm, Mandya, Karnataka. The plant's materials such as Malabar Spinach, Purslane, and *A. vera* were collected from field and flax seeds and fenugreek seeds were purchased from Shop, Mysore. The plant materials were taxonomically identified and authenticated at Regional Ayurveda Research Institute for Metabolic Disorder, Bengaluru, India.

### Extraction of mucilage

The extraction of mucilage from the plant sources was carried out as per method [25]. The mucilage from *A. vera* was extracted from the leaf by peeling and kept overnight at below 20°C. The slurry/mucilage is obtained by filtering the extract through muslin cloth [26]. Flax seeds and fenugreek seeds were crushed and soaked in 1:5 W/V of water for 6 h and boiled in water bath for 5 h then cooled at below 20°C overnight. The extract was filtered through a muslin cloth to obtain mucilage/slurry [27]. Cleaned leaves and stem of purslane and Malabar spinach plant was chopped into small pieces and soaked in 1:3 W/V of water for 6 h and boiled in water bath for 5 h then cooled below 20°C overnight. The extract was filtered through a muslin cloth to obtain the mucilage/slurry [28,29]. The mucilage was subjected to chemical tests such as Molisch test and Ruthenium red test to confirm its identity [30].

### Preparation of jaggery using mucilaginous clarificants

Five jaggery samples were prepared using *A. vera* mucilage, flax seeds mucilage, fenugreek mucilage, Purslane mucilage and Malabar spinach mucilage as clarificants at a dosage concentration of 0.1%, 0.2% and 0.4% (Table 1). The sugarcane was cleaned and washed to remove dirt and foreign particles from the surface. A two roller power crusher was used to extract the juice and the extracted juice was allowed to settle in a container where lighter fraction comprising of wax, carbon and undesirable solid particles will settle at the bottom. The semi-clear juice

(supernatant) was boiled with the addition of lime (calcium hydroxide) to adjust the pH to neutral [31], at this stage mucilage clarificant was added in 2 to 3 sequence and scum formed during boiling was removed time to time. The boiling is continued until the juice attains a temperature of 118–120°C [32]. The hot syrup was allowed to cool and transferred into molds of different shapes and sizes for solidification. The solid jaggery prepared is stored under dry conditions for further analysis. Similarly, the jaggery was prepared without addition of any clarificant was used as a control for comparing purpose.

### Determination of total phenol content

Total phenol content of jaggery was determined using Folin-Ciocalteu method [33]. One gram of jaggery sample was extracted with 10 mL of methanol:water (50:50, v/v) solution. Then, 1 mL of diluted (0.1 mL sample aliquot and 0.9 mL of distilled water) extract was mixed with 5 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4 mL of aqueous sodium bicarbonate (1 M). The solution was allowed to stand for 15 min and then the absorbance of the solution was measured against a blank at 765 nm in a spectrophotometer. A gallic acid standard curve was prepared using 0, 20, 40, 60, 80 and 100 µg/mL of methanol:water (50:50, v/v). Total phenol values were expressed in terms of the standard reference compound as gallic acid equivalent in milligrams per gram of jaggery.

### Determination of total flavonoid content

Spectrophotometric method using aluminum chloride was used for the determination of flavonoids in jaggery with slight modification [31]. 50 microliter of jaggery sample (10%) was added to 400 µL of methanol. Then, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 1.8 mL of distilled water were sequentially added to the reaction tubes. The reaction mixture was kept at room temperature for 30 min and later the absorbance of the reaction mixture was measured at 415 nm wavelength in a spectrophotometer. A calibration curve was prepared using standard quercetin at different concentrations (20–100 µg/mL in methanol). Total flavonoid content was expressed in terms of the standard reference compound as quercetin equivalent in mg/g of jaggery.

### Reducing power assay

The reducing power of jaggery samples was determined according to the method reported earlier [34]. The jaggery sample (1–10 mg/mL) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then an equal volume of 10% trichloroacetic acid was added to the mixture and then centrifuged at 3000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1% ferric chloride at a ratio of 1:1:2 (v/v/v) and the absorbance of the solution measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

**Table 1: Preparation of mucilage clarificants for 10L Sugarcane raw Juice**

Experiment	Sample code	Mucilage concentration (%)	Quantity (g) of mucilage per 10 L of juice
Control	JNC	No mucilage	-
<i>A. vera</i> mucilage	JAV1	0.1	10
	JAV2	0.2	20
	JAV4	0.4	40
	JFS1	0.1	10
Flax seed mucilage	JFS2	0.2	20
	JFS4	0.4	40
	JFG1	0.1	10
Fenugreek mucilage	JFG2	0.2	20
	JJFG4	0.4	40
	JJPS1	0.1	10
	JJPS2	0.2	20
Purslane mucilage	JPS4	0.4	40
	JMS1	0.1	10
	JMS2	0.2	20
Malabar spinach mucilage	JMS4	0.4	40

*A. vera*: *Aloe vera*

### Antibacterial property

Antibacterial activity was determined by agar well diffusion assay [35]. For the study Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*) were selected. Test cultures were grown on Luria Bertani Agar slants and culture densities were prepared using sterile phosphate buffer saline pH 7.2. Culture density was adjusted to 0.12–0.14 absorbance at 530 nm. Each test culture (0.4 mL) was mixed with molten cooled sterile Mueller-Hinton agar butt (20 mL) and was poured into 9 cm Petri plates. Wells of diameter 8 mm were bored in the agar plates. 50  $\mu$ L of Jaggery sample prepared in sterile distilled water was added to each well. The plates were incubated for 24 h at 37°C. Results were interpreted by measuring the size of the zone diameter of inhibition surrounding the wells on the agar plates.

### Anthelmintic activity

The anthelmintic activity of jaggery prepared using plant mucilage clarificants was determined according to the method described with slight modification [12]. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Briefly, 10 g of jaggery dissolved in 20 mL of distilled water was taken in 9 cm Petri dish and six earthworms of approximately equal size were placed in it. Piperazine hexahydrate (10 mg/mL) was used as the reference standard and distilled water as a control. All the test solutions and standard drug solution were prepared freshly before starting the experiments. The time taken for paralysis when no movement was observed even when the worms were shaken vigorously was reported. Similarly, time for the death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water. All the results were expressed as mean  $\pm$  SD (n=6).

### Statistical analysis

All the experiments were carried out in triplicates, and the results were expressed as the mean  $\pm$  standard deviation (n=3).

## RESULTS AND DISCUSSION

### Antioxidant activity of jaggery prepared using plant mucilage clarificants

Results of total phenolic and flavonoids content in jaggery samples prepared using plant mucilage clarificants are shown in Figs. 1 and 2, respectively. The total phenolic content in test jaggery samples was higher than the control jaggery. A good positive correlation was observed between the concentration of plant mucilage clarificants and total phenol content. Among the test jaggery samples, jaggery prepared using *A. vera* at 0.4% concentration indicated highest phenolics content (3.68 $\pm$ 0.02) while the Malabar spinach treated jaggery had the least phenolics content (3.51 $\pm$ 0.01).

The flavonoid content of tested jaggery samples was higher than control jaggery at 0.2 and 0.4% mucilage concentrations. *A. vera*-treated jaggery indicated marginally higher flavonoid content compared to other plant mucilage treated jaggery samples. However, among the test jaggery samples except *A. vera*-treated jaggery, no significant differences in flavonoid content were evident.

The presence of higher total phenolic and flavonoid content in plant mucilage clarificants treated jaggery compared to control may be due to minimal chemical processing in the preparation of jaggery and maximal removal of scum, reduced preparation time followed by good jaggery yield which could have played a major role in retaining good amount of polyphenols.

Further, the antioxidant activity of jaggery prepared using plant mucilage clarificants was evaluated for reducing power. Reducing power assay provides a measure of compounds ability to donate electrons and reduce the oxidized intermediates formed in the peroxidation process. The assay was based on the reduction of the Fe<sup>3+</sup>-ferricyanide complex which was monitored by measuring the formation of Perl's blue at

700 nm. The increased absorbance at 700 nm indicated the presence of reducing power [36]. In Fig. 3 A-C, the jaggery samples exhibited *in vitro* ferric reducing potential in a dose-dependent manner. The absorbance of test jaggery samples increased at 700 nm compared to jaggery control. The reducing powers of jaggery samples at 0.1, 0.2, and 0.4% mucilage clarificants were in the order, JAV > JFG > JFS > JPS > JMS > JNC. At 10 mg/mL concentration, an absorbance unit of 1.93 and 1.80 was observed for jaggery prepared using 0.4% *A. vera* and fenugreek seed mucilage (Fig. 3c). Since the test jaggery samples exhibited higher reducing power compared to jaggery without clarificants, it is certain that the increased reducing power may be attributed to the addition of plant mucilage extract for clarification which may contain antioxidant phytochemicals. The reducing power serves as a significant indicator of antioxidant activity [13,37]. The biomolecule, especially polyphenolic compounds, has been credited for the health-promoting properties such as prevention of chronic cardiovascular diseases [38]. The reducing power of phenolic compounds in sugarcane juice as well as in brown sugar has been reported [39,40]. The Millard reaction products may also contribute to the observed reducing power but to a lesser extent [41].

Jaggery shows antioxidant properties probably due to the presence of phenolic acids, flavonoids, and other phenolic compounds [9,42-45]. The health effects of antioxidants in particularly of polyphenols have still not been scientifically established as the European Food Standards Agency [46]. Jaggery being a least processed sugar retains phenolics and other phytochemicals with potent biological activities such as antioxidant, cytoprotective and anthelmintic activity as reported in the literature [7,12,19].

Sugarcane juice extract contains a wide range of phenolic compounds such as flavonoids [42] and phenolic acids [41] such as apigenin, luteolin, triclin, caffeic, cinnamic acid, sinapic acids and chlorogenic acid. Jaggery showed relatively significant antioxidant activity equivalent

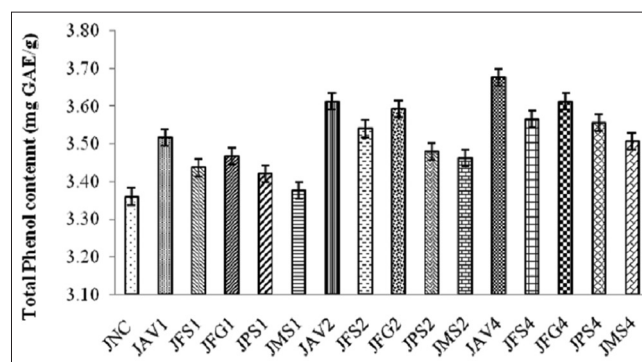


Fig. 1: Total phenolic content in jaggery prepared using plant mucilage clarificants

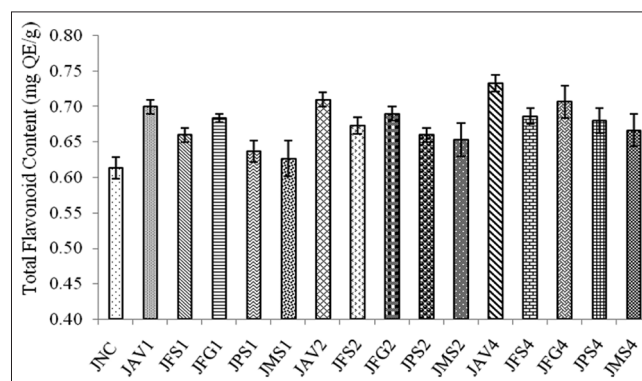


Fig. 2: Total Flavonoid contents in jaggery prepared using plant mucilage clarificants

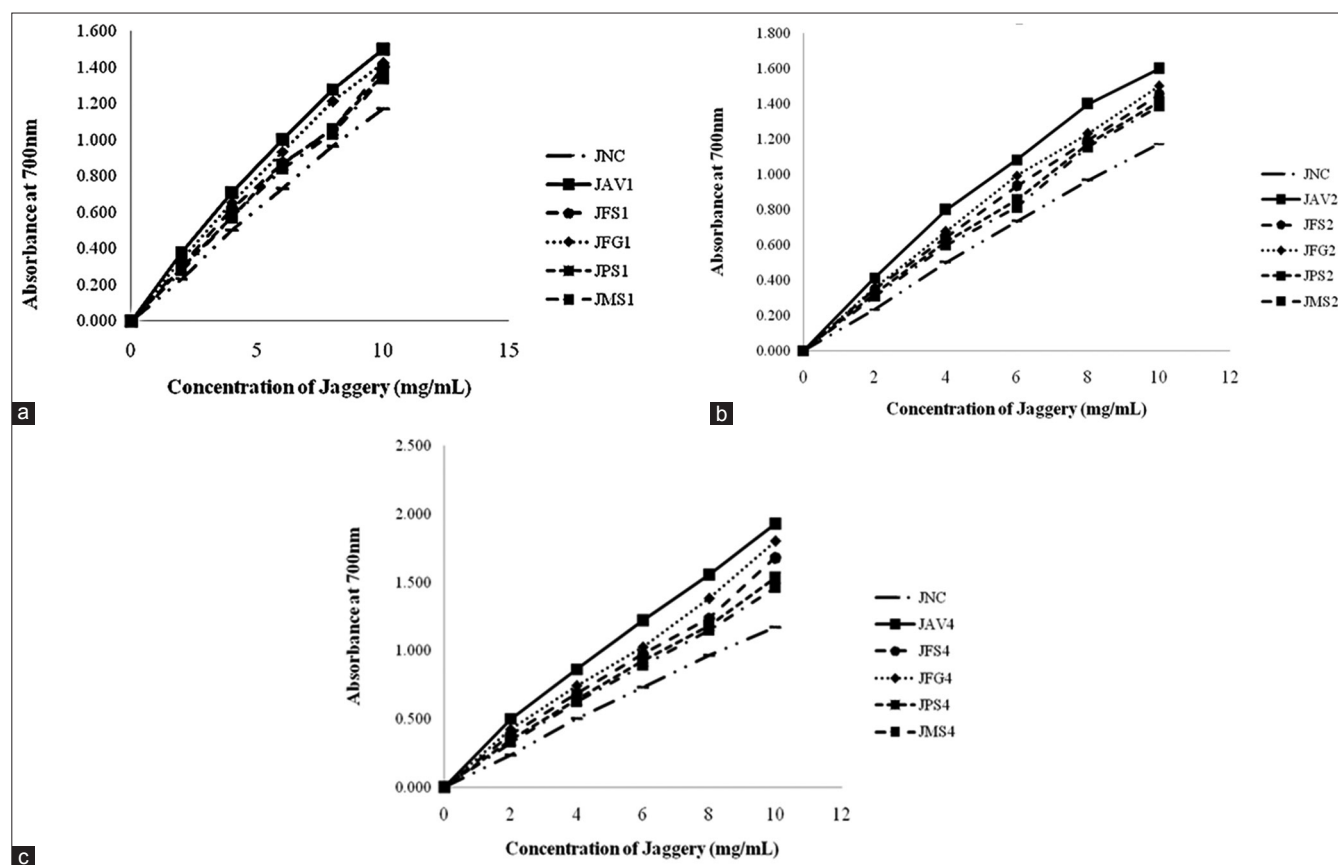


Fig. 3: (a) Reducing power of jaggery prepared using plant mucilage clarificants at 0.1% concentration, (b) reducing power of jaggery prepared using plant mucilage clarificants at 0.2% concentration, (c) reducing power of jaggery prepared using plant mucilage clarificants at 0.4% concentration

to BHT in earlier reports [47]. The traditional practice of adding antioxidants during processing can still play a very important role as added compounds have additional potential for enhancing endogenous antioxidant systems. In the present investigation, jaggery processing using plant mucilage as a clarificants led to the synergistic addition of both total phenolic and flavonoids content resulting in the increased antioxidative potential of jaggery. Free radicals are implicated in the etiology of many human diseases and antioxidant could act as potential therapeutic agents [48]. Hence, the combination of nutritional and medicinal benefits of jaggery prepared using plant mucilage as clarificants can be a potential nutraceutical food.

#### Antibacterial activity of jaggery prepared using plant mucilage as clarificant

The antibacterial activity of jaggery prepared using plant mucilage as clarificant along with control was determined by measuring the diameter of inhibition zone, and the results are indicated in Table 2. Among the test jaggery samples prepared using plant mucilage as clarificant, only *A. vera* ( $34.0 \pm 2$  mm) and fenugreek ( $32.0 \pm 1.00$  mm) at 0.4% mucilage concentration were effective in inhibiting the growth of Gram-positive bacteria compared to control (Table 2). Against Gram-negative bacteria *A. vera* ( $25.7 \pm 0.58$  mm), fenugreek ( $24.3 \pm 0.58$  mm) and flax seeds ( $24.3 \pm 0.58$  mm) treated jaggery significantly inhibited growth compared to other two mucilage clarificants and control. Based on the diameter of the inhibition zone, it was observed that Gram-positive bacteria were more sensitivity to the experimental jaggery samples than Gram-negative bacteria. The antibacterial activity may be attributed to the polyphenols and antioxidant properties of the jaggery [49]. The present study showed that antibacterial activity is closely related to the concentration of phenolic and flavonoids and thus to the antioxidant capacity of the jaggery prepared using plant mucilage clarificants.

#### Anthelmintic activity of jaggery prepared using plant mucilage clarificants

Helmintiasis is recognized as a major problem in livestock production throughout the tropics and also for human health. The parasitic gastroenteritis infection caused by several species of intestinal helminths results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity [50]. Chemotherapeutics has remained as the cornerstone for the treatment of helminthic infection due to development of problems such as resistance to drug, chemical residues and toxicity, cost expensive, non-availability, and non-adaptability of drugs in remote areas [51,52].

*In vitro* anthelmintic activity of jaggery derived from plant mucilage clarificants indicated significant decrease in paralytic and death time of helminths. Control jaggery (paralytic time  $36.6 \pm 1.14$  and death time  $47.6 \pm 0.548$ ) and *A. vera* (JAV4) plant mucilage clarificants at 0.4% treated jaggery (paralytic time  $28.6 \pm 1.14$  and death time  $39.6 \pm 1.14$ ) exhibited anthelmintic activity at 0.5 g/mL concentration and the death time of worm was found to be 47.6 min in control versus 39.6 min in test jaggery as tabulated in Table 3. Standard drug piperazine hexahydrate killed earthworm in 28.4 min at 10 mg/mL concentration. However, distilled water (negative control) did not show any effect on helminths.

The predominant effect of piperazine hexahydrate on the worm caused a flaccid paralysis resulting in the expulsion of the worm by peristalsis. Piperazine hexahydrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability leading to muscle relaxation and flaccid paralysis. There are reports to indicate anthelmintic activity of cane jaggery [12]. Similar results were observed in our study at 0.4% concentration of plant mucilage clarificants of *A. vera* (JAV4) treated jaggery samples.

Table 2: Antibacterial activity of Jaggery prepared using plant mucilage clarificants

Sample	Diameter of inhibition zone (mm)* against Gram-positive ( <i>B. subtilis</i> )	Diameter of inhibition zone (mm)* against Gram-negative ( <i>E. coli</i> )
JNC	27.0±1.00	21.0±1.00
JAV1	30.3±0.58	22.0±1.00
JFS1	29.3±0.58	21.0±1.00
JFG1	30.3±0.58	22.3±1.15
JPS1	29.0±1.00	21.0±1.00
JMS1	28.3±0.58	20.7±1.15
JAV2	30.3±1.53	23.0±1.00
JFS2	30.3±0.58	22.3±1.15
JFG2	30.0±1.00	23.7±0.58
JPS2	29.0±1.00	22.7±1.53
JMS2	28.3±1.53	23.3±0.58
JAV4	34.0±2.00	25.7±0.58
JFS4	29.7±0.58	24.3±0.58
JFG4	32.0±1.00	24.7±1.53
JPS4	29.0±1.73	22.3±1.53
JMS4	29.7±1.53	23.7±0.58

\*Including size of well (8 mm). Values are mean±SD (n=3). *B. subtilis*: *Bacillus subtilis*, *E. coli*: *Escherichia coli*

Table 3: Antihelmintic activity of jaggery prepared using plant mucilage clarificants

Sample	Paralytic time (min)	Death time (min)
Positive control	21.6±0.548	28.4±0.548
Negative control	-	-
JNC	36.6±1.140	47.6±0.548
JAV1	32.6±1.140	42.6±1.140
JFS1	34.6±1.140	44.6±1.140
JFG1	33.6±1.140	42.6±1.140
JPS1	35.6±1.140	45.6±1.342
JMS1	35.4±1.517	46.6±1.342
JAV2	30.8±1.304	40.6±1.140
JFS2	32.2±0.837	43.6±1.140
JFG2	31.8±1.304	42.6±1.140
JPS2	33.6±1.140	44±0.707
JMS2	33.6±1.140	44.6±1.140
JAV4	28.6±1.140	39.6±1.140
JFS4	31.6±1.140	41.6±1.140
JFG4	30.6±1.140	40.6±1.140
JPS4	32.4±1.140	42.6±1.140
JMS4	32.4±1.140	43.6±1.140

Values are mean±SD (n=6)

Acidic pH level of natural sweeteners may prevent the growth of many helminths and natural sweeteners have a saturated mixture of saccharides. This mixture has a low water activity and hence water unavailable for worms and creates poor environment for their growth. In addition, total phenol content was higher in 0.4% plant mucilage clarificants of *A. vera* (JAV4) derived jaggery and polyphenolic compounds are known to have antihelmintic activity [53]. Phenolic compounds may interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation [54]. The important effect of tannins on helminthic life is that they bind with free proteins in the gastrointestinal tract of the host animal and also with glycoprotein on the cuticle of parasite and results in the death of parasite [55]. Flavonoids and terpenoids of plant extract were also reported to have antihelmintic properties [56,57]. The polyphenols content and flavonoids of sugarcane alone in jaggery may have contributed to the observed antihelmintic effect against earthworm.

## CONCLUSIONS

Jaggery samples prepared using plant mucilage clarificants from *A. vera*, flax seed, fenugreek, purslane and Malabar spinach sources including control (without clarificants) were evaluated for total phenolic content, total flavonoid content and reducing power. In addition, the antibacterial activity and antihelmintic activity were also carried out.

Results indicated a dose-dependent increased total phenol content and reducing power in test jaggery compared to control. *A. vera*-treated jaggery was very effective in inhibiting Gram-positive and Gram-negative bacteria followed by fenugreek treated jaggery. Further, *A. vera*-treated jaggery had better paralytic as well as death time against roundworms *in vitro* compared to control and jaggery prepared without clarificants. Overall, the jaggery prepared using plant mucilage as clarificants had higher total phenols and flavonoids and exhibited enhanced reducing power, antibacterial activity and antihelmintic activity. Thus, the application of plant mucilage during sugarcane juice clarification may be recommended as an alternative to synthetic chemical clarificants for the production of nutraceutical jaggery.

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## AUTHOR'S CONTRIBUTION

The present research idea was conceived and supervised by Harishnayaka. M.A. The jaggery samples were prepared using plant mucilage clarificants by Lava Chikkappaiah supported by Venkatesh. K.S. The bioactivity experiments and statistical analysis were carried out by Lava Chikkappaiah, Gunashree. B.S, and Sudharshan. S. Manuscript preparation was carried out by Lava Chikkappaiah under the supervision of Harishnayaka. M.A.

## CONFLICTS OF INTEREST

We declare that there are no conflicts of interest.

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