

Original Research Article

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***Decalepis hamiltonii* (Wight & Arn.) Fortified Jaggery: Its Physico-chemical Characterization and Studies on its *In vitro* Biological Activities**

C. Vinutha¹, M. A. Harish Nayaka^{2*}, G. Sowmyashree³ and Dinesh Kumar⁴

¹Department of Biochemistry, College of Agriculture, University of Agricultural Sciences, Dharwad, Hanumanamatti, Haveri-581115, Karnataka, India

²Department of Sugar Technology, Sir M. Visvesvaraya Post Graduate Centre, University of Mysore, Mandya-571402, Karnataka, India

³Department of Biochemistry, Mount Carmel College Autonomous, Bengaluru-560052, Karnataka, India

⁴Department of Chemistry, Bipin Bihari Science PG College, Jhansi-284001, Uttar Pradesh, India

**Corresponding author*

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Jaggery fortified with *Decalepis hamiltonii* (Swallow root) at 0.05 %, 0.1 % and 0.2 % (w/v) concentrations using Co 86032, Co419 and Co 62175 varieties sugarcane juice studied for physicochemical characteristics and *in-vitro* biological activities viz., antioxidant, antilipoxygenase and antihelmintic activities. Physico-chemical analysis of fortified jaggery showed dose proportional reduction in colour, filterability and increased turbidity, insoluble solids, total phenol, protein and flavonoid contents irrespective of sugarcane varieties. DPPH radical scavenging activity and reducing power potential of *D. hamiltonii* fortified jaggery indicated higher antioxidant potential. At 0.2% *D. hamiltonii* concentration, jaggery of Co 86032, Co 419 and Co 62175 varieties indicated an EC₅₀ of 3.139, 3.048 and 3.166 mg/mL, respectively for radical scavenging activity and exhibited 5.23, 34.47 and 11.43% increased reducing power ability compared to control. *D. hamiltonii* fortified jaggery showed enhanced lipoxygenase inhibitory effect in all cane varieties. At 0.05% *D. hamiltonii* fortification, jaggery of Co 86032, Co 419 and Co 62175 varieties showed lipoxygenase activity inhibition with an EC₅₀ of 0.33, 0.31 and 0.32 mg/mL, respectively. Further, positive correlation was observed between total phenolics and DPPH radical scavenging activity ($r = 0.909, 0.894$ and 0.992) and between total phenolics and lipoxygenase inhibitory activity ($r = 0.967, 0.894$ and 0.964) for Co 86032, Co 419 and Co 62175 jaggery, respectively. Antihelmintic activity was evident in both control and fortified jaggery samples. *D. hamiltonii* fortified jaggery might serve as value added natural sweetener for better health wellness.

Introduction

The past decades has seen for significant outbreak in search of traditional medicine for

primary health care and health promotions. Aerobic organisms normally generate reactive oxygen species (ROS) during cellular respiration, an important metabolic

phenomenon to fuel biological activities. The generation of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$) is an inescapable consequence during metabolic respiration in body. So formed free radicals are very unstable and react with bodily chemical groups or substances like DNA, proteins and lipids to cause oxidative destruction (Lu *et al.*, 2010; Craft *et al.*, 2012) leading to cell or tissue injury. Antioxidants are heterogeneous molecules that prevent, protect or delay the extent of oxidative injury to the target biomolecules (Halliwell, 2007) by terminating oxidation chain reactions. Interestingly, majority of aerobic organisms have coevolved with oxidative defense systems to combat ROS mediated oxidative damage. The defense mechanism includes antioxidant enzymes like superoxide dismutase, catalase, peroxidase and glutathione peroxidase, and non-enzymatic antioxidants like vitamin C, vitamin E, glutathione, etc., to protect cells and tissues against ROS harm. Various stress conditions and environmental exposures like tobacco smoke, radiations and other chemical contaminants normally leads to uncontrolled formation of ROS in the body. When there is an imbalance between ROS production and neutralization by body's antioxidant defense systems lead to development of many oxidative stress related degenerative diseases including diabetes (Robertson and Harmon, 2006), cancer (Valko *et al.*, 2006), cardiovascular diseases and aging (Braca *et al.*, 2002; Singh and Jialal, 2006). Antioxidant either synthesized naturally in organisms or supplemented through diet quenches free radicals and decreases the threat of oxidative damage (Knekt *et al.*, 1996). Many synthetic antioxidants (i.e, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-Butyl hydroquinone (TBHQ)) have used in food industries since decades as a food preservative to prevent oxidative deterioration. However, most of the

regulatory guidelines questioned for its toxicity (Grice, 1988; Wichi, 1986; Wang *et al.*, 1998; Guan *et al.*, 2006). There is an inverse relationship between dietary supplementation of antioxidant rich food and incidence of oxidative mediated human diseases (Sies, 1993) and trusted that higher intake of dietary antioxidant combats against the frequency of oxidative stress diseases (Ames *et al.*, 1993). Hence, greater attention received in search and research of antioxidants found naturally in dietary and medicinal plant resources.

The Father of Medicine, Hippocrate's quotes "Let food be thy medicine and medicine be thy food" is the origin of the concept of "Medicine and Food are isogonics" and model of nutraceuticals or functional foods. The term Nutraceuticals, a class of natural products is a hybrid of nutrition and pharmaceutical that serves as a connecting bridge between food and drugs (Adelaja and Schilling, 1999). The use and value of nutraceuticals in food has a long history and long been renowned for health promotions with little scientific evidence of nutrition and medicinal benefits. Currently, nutraceuticals has emerged as a potential food product in the market (Dillard and German, 2000) as the consumers are more alert and eager to know the advanced functional foods and its nutritive significance. The ancient system of Indian medicine (Ayurveda) has documented for the medicinal value of many plants and herbs. Plant diet including herbs, spices, tea, fruits, vegetables, coffee and cacao contribute for total antioxidant capacity and recognized for the beneficial effect on human health (Denys, 2013). In fact, some plants are rich sources of phenolic compounds and phytochemical those shown to scavenge free radicals and exhibit other biological activities (Rice- Evans *et al.*, 1995; Brown and Rice-Evans, 1998) to modulate oxidative stress-related effects. The phytochemicals present in plant-rich diet are

non-toxic that attributes disease prevention and health promotion potentiality. Plant derived antioxidants gained consideration in food industries and pharma-industries for its ability to preserve foodstuffs by preventing oxidation and protect against oxidative damage in biological systems owing to its therapeutic properties and enhancing well-being. There is great deal of search of newer natural bioactive components with health endorsing potential in plant resources.

Sugarcane (*Saccharum officinarum*) is a commercial plant world widely cultivated mainly for manufacture of table sugar (sucrose) and ethanol fermentation. In addition to sucrose, sugarcane also contains various phytochemicals including phenolic compounds that ascribed health-promoting effects (Fontaniella *et al.*, 2003; Duarte Almeida *et al.*, 2006). Jaggery is a non-conventional natural sweetener man-made by concentrating thick syrup of sugarcane juice via heating. Various ayurvedic preparations added jaggery as a 'wholesome sugar' for its preserved good sweetness and health beneficial components. Jaggery is a least processed sweetener retains majority of the phytochemicals present in sugarcane juice than white sugar (table sugar), a pure sucrose. The phytochemicals present in jaggery recognized for various biological activities and health benefits. *In vivo* studies documented for the protective role of jaggery on lung damage in rats caused by particulate matter (Sahu and Saxena, 1994) and also reduced the incidence of chromosomal aberrations in rats when fed with jaggery (Nrashant *et al.*, 2008). Literature studies revealed that dietary supplementation of jaggery and its extracted phenolic compounds reduced the development of arteriosclerosis in Japanese quails (Okabe *et al.*, 2009) and exhibited various biological activities viz., antioxidant, cytoprotective and antihelmintic activity (Harish Nayaka *et al.*, 2009; Prasad *et*

al., 2010). There is less scientific evidence on nutraceutical role of jaggery in various food or ayurvedic preparations and fortification of jaggery as a functional sweetener to promote both nutrition and medicinal significance. Few reports documented for jaggery fortification with naturally available vitamin-C using Indian gooseberry (Anwaar and Singh, 2010), preparation of jaggery chocolates with coaca and coffee powder enrichment (Khan Chand *et al.*, 2011) and neem fortified jaggery for enhanced antioxidant ability (Vinutha *et al.*, 2014).

Herbs and spices are cuisine dishes during food preparations since ancient times to enhance its aroma, flavor, color, astringency and other sensory attributes. Apart from sensory effects, herbs and spices also credited potential health supporting effects by exhibiting antioxidant, anti-inflammatory, antibacterial, antimicrobial activities and served as natural food preservatives (Smid and Gorris, 1999). *Decalepis hamiltonii* well known as swallow root or Makaliberu grow wild as a climbing shrub in the forests of peninsular India. It belongs to the family Asclepiadaceae. *D. hamiltonii* roots have used for making pickles, juices, beverages and in many ayurvedic preparations (Nayar *et al.*, 1978) for its alleged health stimulating properties. *D. hamiltonii* finds use as a flavoring agent, appetizer, blood purifier and preservative. *D. hamiltonii* root extracts exhibited potent antioxidant, antimicrobial, cytoprotective, anti-ulcer and gastro-protective activity (Srivastava *et al.*, 2006a, Srikanta *et al.*, 2011 and Devarajan *et al.*, 2002). The present investigation aims to prepare nutraceutical jaggery sweetener by fortification with *D. hamiltonii* extract and to study the physico-chemical characterization, *in vitro* antioxidant activity, antilipoxygenase activity and antihelmintic activity of *D. Hamiltonii* fortified jaggery of three different sugarcane varieties.

Materials and Methods

Chemicals

All chemicals and solvents used in the experiment were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Himedia Laboratories, Mumbai, India and Trolox from Biomol Research Labs, Inc., USA. Other chemicals like Coomassie Brilliant Blue G-250, Gallic acid, Linoleic acid, Quercetin, Trichloroacetic acid (TA), Butylated hydroxytoluene (BHT) and Bovine serum albumin (BSA) procured from SISCO Research Laboratories Pvt. Ltd., Mumbai, India.

Plant material acquisition

Twelve months old sugarcane varieties namely Co 86032, Co 419 and Co 62175 cultivated on with similar management regimes were obtained from Zonal Agricultural Research Station, V. C. Farm, Mandya 571405, Karnataka. Fresh *Decalepis hamiltonii* roots purchased from a local vegetable market (K R Market) at Mysore, Karnataka, India and were cut into small pieces, air-dried and ground to fine powder.

Preparation of *D. hamiltonii* fortified jaggery

D. hamiltonii fortified jaggery were prepared following the method described by Jagannadha Roa *et al.*, (2007). Briefly, Jaggery fortification with *D. hamiltonii* was made at concentrations viz., 0.05%, 0.1% and 0.2%, w/v using Co 86032, Co 419 and Co 62175 varieties sugarcane juice. Milk of lime [Ca(OH)₂] added to juice to adjust the pH 6.6. Juice mixture initially boiled for 10 minutes and filtered using muslin cloth. The filtrate juice continued boiling to thick syrup until striking temperature attained. The thick syrup was air cooled and molded. Jaggery prepared

without addition of *D. hamiltonii* served as control. All the jaggery samples were stored at 4°C in a sealed plastic container for further analysis.

Physico-chemical characterization

pH, Turbidity, Filterability and Insoluble solids

pH, Turbidity, Filterability and Insoluble solid content of *D. hamiltonii* fortified jaggery were evaluated following methods described earlier (Guerra and Mujica, 2010). Briefly, jaggery solution (5% w/v) analysed for pH using pH meter (Systronics India Pvt. Ltd., India) and turbidity measured by the percentage transmittance at 720 nm in visible spectrophotometer (Systronics India Ltd. Gujarat, India).

Filterability of jaggery solution was evaluated based on the rate of filtration and pure sucrose (28⁰ Brix) served as reference standard. Jaggery and sucrose solutions filtered through Whatman No. 1 filter paper and the percentage of filterability was calculated using the ratio of filtrate volumes. The residue on the filter paper was dried, weighed and analyzed for insoluble solid content of jaggery, and its result was expressed in percentage on dry weight basis.

Color, Water Activity (a_w), moisture and ash content

The color of jaggery in 5 % (w/v) solution was appreciated based on optical density at 540 nm using visible spectrophotometer as defined by Mandal *et al.*, (2006). Water activity was determined using a water activity meter (Novasina, Switzerland). Moisture and ash content of jaggery was measured following standard protocols of official AOAC (Helrich, 1990).

Reducing sugar and non-reducing sugar (Sucrose)

The reducing sugar content of jaggery was estimated by Fehling's method (Lane and Eynon, 1923). Briefly, N/2 jaggery solution (13g in 100 mL) was titrated against Fehling's solution using 1% methylene blue as internal indicator under hot condition and Dextrose (0.5% solution) used as reference standard. Non-reducing sugar (sucrose) content of jaggery was evaluated by ICUMSA method using Double R. Polarimeter (Anon 1999).

Total protein content

The total protein content of jaggery was quantified by the Bradford's method (Bradford, 1976). Briefly, a sample aliquot of 100 μ L (3.5 mg jaggery) was added to 900 μ L of Bradford reagent and the color absorbance recorded at 595 nm using spectrophotometer. Bovine serum albumin (BSA) was used as a reference standard and the protein content of jaggery was stated in terms of mg of BSA equivalent/g.

Total phenol content

The total phenol content of jaggery was estimated spectrophotometrically through Folin-Ciocalteu's method (Singleton *et al.*, 1999). A sample aliquot of 50 mg/mL of jaggery, 1 mL of Folin-Ciocalteu reagent (1:2, v/v) and 2 mL of 10 % sodium carbonate were sequentially added, incubated for an hour at room temperature and the absorbance was noted at 765 nm. Gallic acid was used as a standard. The total phenolic content of jaggery was expressed as mg of Gallic acid equivalent (GAE)/g.

Total flavonoid content

The total flavonoid content of jaggery was determined according to aluminium chloride

method (Chang *et al.*, 2002). A sample aliquot 50mg/mL of jaggery, 50 μ L of 10 % aluminium chloride and 50 μ L of 1M potassium acetate were serially added, incubated for 30 min at room temperature and the optical density recorded at 415 nm. Quercetin was used as a standard and total flavonoid content was expressed in μ g of quercetin equivalent (QE)/g.

***In-vitro* biological activities**

***In-vitro* antioxidant activity**

DPPH radical scavenging activity

The DPPH radical scavenging activity of jaggery was quantified following the method of Yamaguchi *et al.*, (1998). Accordingly, a sample aliquot of jaggery at 10-50 μ g/mL and standard antioxidant at 2-10 μ g/mL was made up to 200 μ L using distilled water. 1 mL of 0.1 mM DPPH was added. The reaction mixture incubated at room temperature in dark for 20 min and the absorbance was recorded against control at 517 nm. The ability to scavenge DPPH radical was calculated and the result expressed as an effective concentration (EC₅₀) for 50% DPPH radical scavenging activity.

$$\text{DPPH Radical Scavenging ability (\%)} = \left[\frac{A_{\text{Control}} - A_{\text{sample}}}{A_{\text{Control}}} \right] \times 100$$

Reducing power assay

The reducing power of jaggery was determined following the method of Yen and Chen (1995). Aliquots of jaggery (1-5 mg/mL) and standard antioxidant Trolox (10-50 μ g/mL) was mixed with an equal volume of 0.2 M Phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The reacting mixture kept warm at 50 °C for 20 min. Further, an equal volume of 10 % trichloroacetic acid added and centrifuged at 3000 g for 10 min.

The supernatant was mixed with distilled water and 0.1 % FeCl₃ at a ratio of 1:1:2 (v/v/v), and the absorbance was measured at 700 nm.

***In-vitro* Antilipoxygenase activity**

Enzyme extraction from soybean

Lipoxygenase enzyme was extracted from soybean following the method described by Vineet Kumara *et al.*, (2006) with slight modification. Briefly, 10 g of soybean seed soaked overnight homogenized at 0-4 °C in 0.2 M phosphate buffer (pH 6.8) for 20 minutes using micro-tissue homogenizer. The homogenate centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant used as crude extract for assaying lipoxygenase activity. The protein content of the crude soybean extract determined by Bradford's method as described earlier.

Lipoxygenase activity

Lipoxygenase activity was analysed following the method described by (Naidu, 1995). Briefly, 500 µL of crude enzyme extract was made up to 2 mL using 50 mM Tris buffer, pH 7.4 and 1mL of 50 µM linoleic acid was added. The increase in absorbance of the reaction mixture monitored at 234 nm wavelength against the blank. One unit of enzyme was equivalent to the enzyme concentration that caused an increase in absorbance of 1.0 per min at 234 nm. The enzyme activity was recorded as µmoles of hydroperoxide formed per minute per µmole of enzyme per volume.

Lipoxygenase inhibitory activity

Lipoxygenase inhibitory activity of jaggery was analyzed following the method described by Naidu, (1995). An aliquot of jaggery sample (100-500 µg) and 500 µL of soybean

lipoxygenase enzyme and 2 mL using 50 mM Tris buffer of pH 7.4 taken in test tubes were pre-incubated for 10 minutes at 37 °C. Then, 1 mL of 50 µM linoleic acid substrate was added and mixed well. The absorbance of the reaction mixture monitored at 234 nm in UV spectrophotometer. Indomethacin served as standard inhibitor. The inhibitory effect of jaggery was recorded in terms of effective concentration EC₅₀ values

$$\text{Lipoxygenase Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100]$$

***In-vitro* antihelminthic activity**

Anti-helminthic activity of fortified jaggery was determined according to the method described by Prasad *et al.*, (2010). The assay performed using adult Indian earthworms (*Pheritima posthuma*) for their resemblance both anatomical and physiological with the intestinal parasitic roundworm infecting gastro-intestine of human beings. Briefly, six earthworms of equal size placed in petri-dishes containing fortified jaggery solution (10g/20 mL). Piperazine-hexahydrate (10 mg/mL) used as standard drug and distilled water as control. The Anti-helminthic activity of jaggery was performed in triplicate trials. The time taken for paralysis (noted when no movement observed except when the worms were shaken vigorously) and the death time of worms (noted ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water, 50 °C) were recorded.

Statistical analysis

All the experiments were carried out in triplicates (n = 3) and the results were represented as mean ± standard deviation (SD) using Microsoft Excel software. IBM SPSS Statistical software version 19.0 was used to analyze the results.

Results and Discussion

Physico-chemical characterization of *D. hamiltonii* fortified jaggery

The data of physical characteristics of *D. hamiltonii* fortified jaggery were presented in Table 1. The pH of *D. hamiltonii* fortified jaggery and its control were slightly acidic and in the range of 5.59 to 5.84 that was in conformity as reported by Guerra and Mujica (2010). Ecuadorian technical standard (2002) has recommended pH 5.9 for jaggery. Turbidity measures the degree of transparency of solution and the presence of suspended particulates. Turbidity of jaggery solution was recorded spectrophotometrically at 720 nm and results indicated that jaggery fortification with *D. hamiltonii* displayed higher turbidity of three-sugarcane variety jaggery tested. At 0.05% *D. hamiltonii* fortification, jaggery of Co 86032, Co 419 and Co 62175 varieties showed 5.8, 8.1 and 6.1% higher turbidity, respectively than its respective control. Enhanced turbidity of jaggery solution directed greater presence of suspended particulates in jaggery samples. The insoluble solid content of jaggery also showed to increase by 0.4% with decreased rate of filterability about 0.15% for Co 86032 and Co 419 jaggery varieties and 0.46% for Co 62175 jaggery variety. Turbidity of the solution is directly proportionate to insoluble solid content and inversely related to rate of filtration of the jaggery samples. Further, the ash content of fortified jaggery differed by only 0.01%. Increased turbidity and higher insoluble solids in fortified jaggery presumed that the phytochemicals from added *D. hamiltonii* spice extract during jaggery making were retained in final product. The observations viz. increased turbidity, insoluble solids content and decreased filterability of fortified jaggery interprets that *D. hamiltonii* dried powder might not be good clarificant to accomplish sugarcane juice clarification.

The color of jaggery was computed spectrophotometrically based on the absorbance of jaggery solution at 540 nm. Color plays a prominent role in consumer's choice and acceptance of jaggery and its market value. The presence of plant pigments like chlorophylls, xanthophylls, carotenes and anthocyanins intensifies dark color to sugarcane juice. Non-enzymatic browning reactions, caramelization and Maillard reactions during juice boiling further deepen dark coloration to juice (Hodge, 1953; Tomasik *et al.*, 1989). Presence of free reducing sugars, both aldoses and ketoses, in particularly fructose contributes ample dark coloration to jaggery during thermo-evaporation (Burton and McWeeny, 1963; Burton *et al.*, 1963). Reduction in color intensity was noticed in jaggery upon fortification with *D. hamiltonii*. At 0.2 % *D. hamiltonii* concentration, jaggery Co 86032, Co 419 and Co 62175 showed 32.2, 24.9 and 27.1% lower color absorbance than their respective controls. Accordingly, jaggery fortification with *D. hamiltonii* diminishes brown color significantly and imparts better coloration to upsurge its consumer's preference and market demand.

The principal factors of keeping quality of jaggery are its moisture content and water activity. Moisture content of jaggery measures the amount of water in terms of percentage whereas water activity (a_w) quantifies free or active water available to support the growth of microorganisms in jaggery. Moisture content of jaggery both control and fortified samples varied slightly and were in the range of 4-6% that was in concurrent as reported by Guerra and Mujica (2010). Bureau of Indian Standards, BIS 1990 stated that the moisture content of freshly prepared jaggery must be 5-7% (Anonymous, 1990). Water activity (a_w) of jaggery was between the values 0.6 and 0.7 that generally benefits for the proliferation of food spoilage

microbes on jaggery (Beuchat, 1981). Thus, proper hygienic practices may be supportive to improve shelf life of *D. hamiltonii* fortified jaggery.

Chemical characteristic of *D. hamiltonii* fortified jaggery were tabulated in Table 2. The chief component of jaggery is non-reducing sugar, sucrose; and glucose being major reducing sugar. Reducing sugar content and sucrose content in both control and fortified jaggery at all *D. hamiltonii* concentrations of tested sugarcane varieties remained unchanged. However, the total protein, phenol and flavonoid contents of fortified jaggery of tested sugarcane varieties significantly increased with dose dependent *D. hamiltonii* addition. The total protein content of jaggery as quantified by Bradford's method was noted between 0.9 and 1.49 mg/g. At 0.2% *D. hamiltonii* fortification, Co 86032, Co 419 and Co 62175 jaggery boosted its total protein content by 23.7, 18.3 and 23.0%, respectively. Further, the total phenol content of Co 86032 and Co 419 fortified jaggery increased by 21.8 & 19.5% and flavonoid content by 55.3 & 65.0%, respectively from their respective control but Co 62175 variety fortified jaggery showed only 2% and 17% higher total phenol and flavonoid content, respectively. Polyphenols are the secondary metabolites of the plant kingdom exhibits various biological activities (Duthie and Crozier, 2000; Ghasemzadeh and Ghasemzadeh, 2011; Ferrazzano *et al.*, 2011). The dietary sources of polyphenols include fruits, vegetables, spices, cereals, plant-derived beverages and natural sweeteners (Scalbert *et al.*, 2005). Our investigation showed that the total phenol and flavonoid contents in jaggery have increased upon addition of *D. hamiltonii* and this enhanced polyphenolic components in fortified jaggery may contribute for its heightened biological activities.

***In-vitro* antioxidant activity of *D. hamiltonii* fortified jaggery**

DPPH radical scavenging and reducing power assays employed to evaluate *in vitro* antioxidant activity of *D. hamiltonii* fortified jaggery. DPPH, a stable free radical used to study the free radical scavenging ability of natural compounds. The principle of the assay includes reduction of absorbance of methanolic DPPH solution at 517 nm after accepting an electron or hydrogen radical from an antioxidant to form non-radical DPPH. The degree of decolourisation of DPPH with an antioxidant is stoichiometric and inversely proportional to radical scavenging ability of that antioxidant (Blios, 1958). Figure-1 demonstrated the DPPH radical scavenging ability of control and *D. hamiltonii* fortified jaggery of three sugarcane varieties studied. The radical scavenging potential of jaggery and standard antioxidant BTH was expressed as EC₅₀ values (half-maximal effective concentration). Lower the EC₅₀ values of an antioxidant indicate its higher free radical scavenging potentiality. Free radical scavenging ability of an antioxidant relies on its hydrogen donation capacity (Chen and Ho, 1995). The results revealed that the free radical scavenging potentiality of fortified jaggery was dose proportional with *D. hamiltonii* added to jaggery during fortification. At 0.2% *D. hamiltonii* concentration, Co 86032, Co 419 and Co 62175 fortified jaggery displayed higher DPPH radical scavenging capacity with EC₅₀ values of 3.139, 3.048 and 3.166 mg/mL, respectively and standard antioxidant BHT had EC₅₀ = 0.008mg/mL, that was only 400 folds lower concentration needed to illustrate free radical scavenging activity than fortified jaggery. However, a good positive correlation ($r = 0.909, 0.894$ and 0.992) was established between the phenolic content and DPPH scavenging activity of *D. hamiltonii* fortified jaggery for Co 86032, Co 419 and Co 62175 varieties, respectively.

Table.1 Physical properties of *D. hamiltonii* fortified jaggery from different sugarcane varieties (Mean ± Standard Deviation)

Sugarcane variety	Co 86032				Co 419				Co 62175			
	<i>D.hamiltonii</i> Concentration (Ctrl)	0 %	0.05 %	0.1 %	0.2 %	0 %	0.05 %	0.1 %	0.2 %	0 %	0.05 %	0.1 %
pH	5.800 ± 0.040	5.840 ± 0.040	5.790 ± 0.040	5.770 ± 0.040	5.690 ± 0.010	5.570** ± 0.010	5.640* ± 0.010	5.690 ± 0.010	5.820 ± 0.020	5.810 ± 0.050	5.640** ± 0.030	5.640** ± 0.020
Colour (OD at 540 nm)	0.798 ±0.003	0.783 ±0.018	0.773** ±0.005	0.541*** ±0.011	0.904 ±0.004	0.894** ±0.001	0.745** ±0.002	0.679*** ±0.002	1.157 ±0.001	1.078* ±0.002	0.975** ±0.003	0.843*** ±0.004
Moisture (%)	5.100 ± 0.04	5.800** ± 0.04	5.600** ± 0.03	5.800** ± 0.02	5.625 ± 0.030	5.100** ± 0.030	6.200** ± 0.030	6.200** ± 0.030	4.800 ± 0.010	4.800 ± 0.030	4.700* ± 0.010	4.000*** ± 0.010
Turbidity (%) Transmittance at 700 nm)	36.20 ± 0.04	34.10** ±0.02	33.10*** ±0.03	31.80*** ±0.04	29.60 ± 0.03	27.20* ±0.03	26.10** ±0.03	24.90*** ± 0.03	19.60 ± 0.05	18.40 ±0.05	16.00** ±0.04	14.70*** ±0.03
Filterability (%)	79.17 ± 0.02	79.03* ± 0.04	78.79** ± 0.03	77.35** ± 0.04	70.83 ± 0.03	70.68* ± 0.02	70.41* ± 0.04	69.99* ± 0.03	64.58 ± 0.02	64.12* ± 0.03	63.85* ± 0.01	63.59** ± 0.02
Insoluble solids (g/100g)	5.340 ± 0.02	5.720** ± 0.02	6.170** ± 0.03	6.750** ± 0.02	7.390 ± 0.02	7.520** ± 0.03	7.590** ± 0.03	7.650*** ± 0.01	7.530 ± 0.02	7.850** ± 0.02	8.130*** ± 0.02	8.280*** ± 0.02
Water Activity, a_w (%)	0.699 ± 0.01	0.714** ± 0.03	0.708* ± 0.03	0.715** ± 0.02	0.679 ± 0.03	0.712** ± 0.02	0.726** ± 0.04	0.729** ± 0.06	0.689 ± 0.03	0.683 ± 0.02	0.679* ± 0.02	0.635* ± 0.03
Ash (%)	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00

t-test:**P*<0.05; ***P*<0.01; ****P*<0.001

Table.2 Chemical properties of *D. hamiltonii* fortified jaggery from different sugarcane varieties (Mean ± Standard Deviation)

Sugarcane variety	Co 86032				Co 419				Co 62175			
	0% Ctrl	0.05%	0.1%	0.2%	0% Ctrl	0.05%	0.1%	0.2%	0% Ctrl	0.05%	0.1%	0.2%
<i>D. hamiltonii</i> Concentration												
Sucrose (%)	71.44 ± 0.01	71.41 ±0.03	71.47* ±0.02	71.42 ±0.04	72.76 ±0.01	72.79 ±0.02	73.77 ±0.03	73.76 ±0.03	73.00 ±0.03	73.28* ±0.03	73.54* ±0.02	73.73* ±0.03
Reducing sugar (%)	17.28 ±0.02	17.34 ±0.05	17.23 ±0.04	17.30 ±0.07	17.09 ±0.01	17.12 ±0.03	17.14 ±0.05	17.09 ±0.03	17.42 ±0.03	17.45 ±0.03	17.42 ±0.03	17.48 ±0.04
Protein (mg/g)	0.93 ± 0.01	1.01* ±0.02	1.09* ±0.02	1.15* ±0.03	1.09 ± 0.02	1.18 ±0.04	1.23* ±0.03	1.29* ±0.03	1.21 ±0.04	1.23 ±0.02	1.41* ±0.01	1.49* ±0.02
Total Phenol (mg/g)	3.16 ±0.04	3.45** ±0.04	3.48** ±0.03	3.85** ±0.04	3.43 ± 0.05	3.87* ±0.04	3.99** ±0.04	4.10*** ±0.02	3.76 ±0.02	3.77 ±0.02	3.79 ±0.02	3.83* ±0.02
Total Flavanoids (mg/ml)	0.47 ±0.02	0.58* ±0.01	0.60* ±0.02	0.73** ±0.02	0.60 ± 0.02	0.73* ±0.04	0.87** ±0.03	0.99** ±0.02	0.65 ±0.02	0.66 ±0.02	0.70* ±0.02	0.76* ±0.02

t-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table.3 Anthelmintic activity of *D. hamiltonii* fortified jaggery from different sugarcane varieties (Mean ± Standard Deviation)

Sugarcane variety	Co 86032 (500mg/mL Jaggery solution)				Co 419 (500mg/mL Jaggery solution)				Co 62175 (500mg/mL Jaggery solution)				Standard Piperazine-hexahydrate (10mg/mL)	Distilled Water
	0% Ctrl	0.05%	0.1%	0.2%	0% Ctrl	0.05%	0.1%	0.2%	0% Ctrl	0.05%	0.1%	0.2%	Positive control	Negative control
<i>D. hamiltonii</i> Concentration														
Paralytic time (min)	16.13 ± 1.20	15.47 ± 0.55	15.45 ± 1.09	14.51 ±1.14	16.45 ±2.10	16.04 ± 1.00	15.58 ± 0.59	15.12 ± 1.02	15.44 ± 1.40	14.58 ± 0.46	14.14 ± 1.08	13.41 ± 1.30	23.08 ± 1.00	-
Death time (min)	20.23 ± 2.20	19.48 ±1.05	19.13 ± 1.08	18.39 ± 0.58	19.44 ± 1.30	19.19 ± 1.14	18.53 ± 1.23	18.16 ±2.07	19.46 ± 1.00	19.13 ± 1.05	18.52 ± 0.57	18.29 ± 1.27	26.58 ± 1.20	-

Fig.1 DPPH radical scavenging activity of *D. hamiltonii* fortified jaggery and Standard BHT (* P < 0.05; ** P < 0.01 & *** P < 0.001)

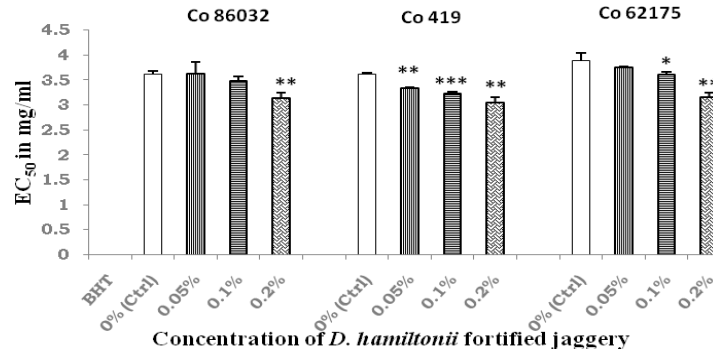


Fig.2 Reducing power of *D. hamiltonii* fortified jaggery and Standard Trolox (* P < 0.05; ** P < 0.01 & *** P < 0.001)

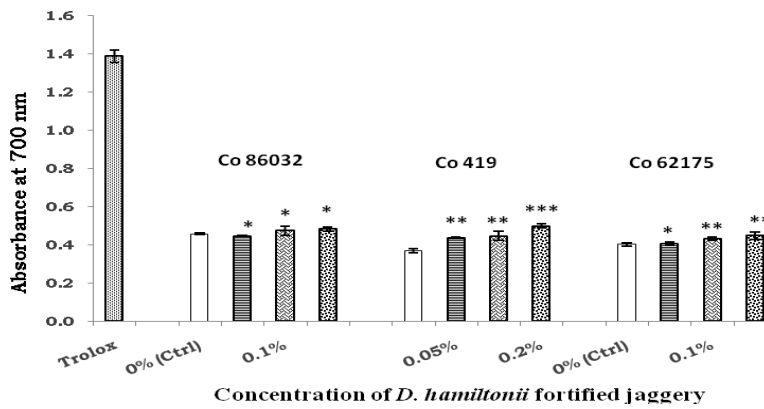
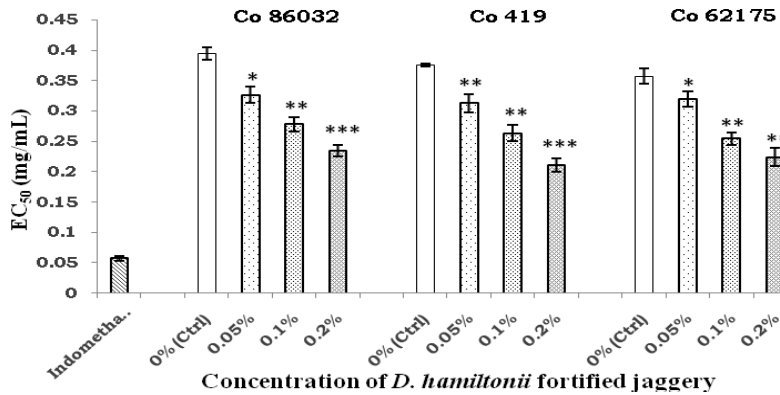


Fig.3 Anti-lipoxygenase activity of *D. hamiltonii* fortified jaggery and Standard Indomethacin (* P < 0.05; ** P < 0.01 & *** P < 0.001)



In addition, the reducing potentiality of an antioxidant quantified by using reducing power assay depends on the capability of an antioxidant to reduce ferric chloride and potassium ferricyanide complex monitored spectrophotometrically at 700 nm.

The rise in absorbance of the reaction mixture was proportionate to reducing power ability. The reducing ability of an antioxidant narrates to its electron transfer capacity and serves as a substantial indicator of its potential antioxidant activity (Meir *et al.*, 1995). Reducing potential of *D. hamiltonii* fortified jaggery and standard Trolox were presented in Figure 2. Fortified jaggery of all sugarcane varieties studied recorded increased absorbance at 700 nm and was dose proportional with *D. hamiltonii* fortification. The recorded data confirmed that Co 86032, Co 419 and Co 62175 fortified jaggery at 0.2% *D. hamiltonii* addition caused enhancement of reducing potential approximately by 5.23, 34.47 and 11.43%, respectively from its control jaggery. On an average, the absorbance at 700 nm of both control and *D. hamiltonii* fortified jaggery samples were nearly three folds lower compared to standard antioxidant Trolox (5mg/mL).

Various plant extracts have found application in food industries as natural preservatives to check lipid oxidation and microbial growth in food. The phenolic compounds present in plants has hydrogen donating capacity and are responsible for inhibition of free radical induced oxidation and hence exhibit antioxidant activity (Yen *et al.*, 1993). Sugarcane juice is abundant with polyphenols and exhibited antioxidant potentiality (Duarte Almeida *et al.*, 2006). Jaggery is prepared by boiling sugarcane juice; most of polyphenols are reserved in final product jaggery. Brazilian panela or jaggery reported to contain antioxidant vitamins viz., Vitamin A

and Vitamin C (Bernal *et al.*, 2004) and various phenolic glycosides present in jaggery attributed for strong free radical scavenging potential than synthetic antioxidant BHT (Takara *et al.*, 2002). Our own earlier studies on jaggery have shown strong DPPH scavenging ability with EC50 = 7.81 µg/mL and reducing power with absorbance of 2.66 at 20 mg/mL (Harish Nayaka *et al.*, 2009). Enrichment of jaggery with spices like ginger and cardamom promoted its antioxidant activity (Harish Nayaka *et al.*, 2015; Vinutha Chandrakanth *et al.*, 2019). The aromatic tuberous root, *D. hamiltonii* well-known for its medicinal properties. Aqueous extracts of *D. hamiltonii* document its strong DPPH scavenging effect and reducing power (Srivastava *et al.*, 2006b).

In this study, fortification of jaggery with *D. hamiltonii* statistically enhanced the total phenol and flavonoid contents in jaggery and marked positive correlation between total phenolic content and antioxidant potentiality. Similar positive correlation between total phenolic content and antioxidant activity in plants are documented (Benzie and Szeto, 1999). An antioxidant-rich natural sweetener may serves as a good source of nutraceuticals and plays a key role in maintenance of health and well-being.

***In-vitro* antilipoxygenase activity of *D. hamiltonii* fortified jaggery**

Most of the oxidative enzymes performing normal biological processes are also involved in pathological conditions. Lipoxygenase enzymes belong to such category are produced in body during pathological conditions and are mainly involved in biosynthetic pathways leading to formation of leukotrienes, a key mediators of allergic response, pain and inflammation. In human tissues, lipoxygenases are produced by immune cells like neutrophils, eosinophils

and by platelets, synovial fluid, lung tissues and bone marrow cells (Steinhilber, 1999). Lipoxygenase catalyzes metabolic oxygenation reaction in cellular system and the resulting metabolites are important bio-regulatory compounds viz., hydroperoxy-eicosa-tetraenoic acids (HPETE), hydroxy-eicosa-tetraenoic acid (HETE), leukotrienes and lipoxins that cause psoriasis, rheumatoid arthritis, glomerular nephritis, cancer, tumour, neuropathic and inflammatory pain (Sircar *et al.*, 1983; Bhattacharjee, 2007). Continued administration of synthetic anti-inflammatory drugs for a long time had adverse side effects and therefore there is a demand to explore natural tactics to reduce the formation of inflammatory mediators through dietary products. *In vitro* lipoxygenase inhibitory activity of *D. hamiltonii* fortified jaggery was analyzed spectrophotometrically using linoleic acid as the substrate for the enzyme lipoxygenase extracted from soybean. The enzyme converts the substrate linoleic acid into conjugated dienes that is monitored with increase in absorbance at 234 nm using UV-spectrophotometer.

The inhibitory effect of fortified jaggery of three tested sugarcane varieties on soybean lipoxygenase activity was represented in Figure 3. The inhibitory effect of jaggery on soybean lipoxygenase was expressed in terms of EC_{50} values (50% inhibitory concentration). The lipoxygenase activity in crude soybean extract noted as 32U. Control jaggery of all three sugarcane varieties evaluated documented 50% inhibitory activity of enzyme at 0.37 mg/mL and reference standard indomethacin showed an EC_{50} of 57.2 μ g/mL. *D. hamiltonii* fortified jaggery appraised greater lipoxygenase inhibitory effect and was proportional to fortified spice concentration. At 0.05% *D. hamiltonii* fortification, jaggery had EC_{50} of 0.33, 0.31 and 0.32 mg/mL for Co 86032, Co 419 and Co 62175 jaggery varieties, respectively.

Further, correlation coefficient between total phenol content and lipoxygenase inhibitory activity of fortified jaggery were calculated. A good positive correlation ($r = 0.967, 0.894$ and 0.964) was recorded for Co 86032, Co 419 and Co 62175 jaggery, respectively between phenolics and enzyme inhibitory action. Plant polyphenols work as crucial regulators of biological oxidation and demonstrated supposedly beneficial inhibitory action of inflammatory enzyme, lipoxygenase. Hence, phenolics application for the management of inflammatory diseases recommended earlier (Sreejayan and Rao, 1996). Lipoxygenases are susceptible to antioxidants; antioxidants inhibit lipoxygenase by scavenging free radicals and thereby, antioxidants are good anti-inflammatory compounds as they impede formation of lipid-hydroperoxide, an intermediate metabolite of lipoxygenase action by scavenging lipid-oxy or lipid-peroxy radicals (Rackova *et al.*, 2007). Further, antioxidants can also hinder lipoxygenase action via non-heme bound-iron chelation (Lin *et al.*, 2001) and iron reduction (Gutierrez-Lugo *et al.*, 2004).

Phytochemicals like phenolics and flavonoids documented to modulate the activity of lipoxygenase (Laughton *et al.*, 1991) by inhibiting lipoxygenase activity and blocking arachidonic acid metabolic pathway. Active principles of spices considered as good inhibitors of lipoxygenase enzyme and showed synergetic inhibitory effect on enzyme activity (Prasad *et al.*, 2004). The results of *in vitro* analyzes of lipoxygenase activity reveal that polyphenols of *D. hamiltonii* has contributed for lipoxygenase inhibitory effect of jaggery of all tested varieties and henceforth, jaggery fortification with *D. hamiltonii* may enrich jaggery with supplementary health benefit to combat inflammation.

***In-vitro* antihelmintic activity of *D. hamiltonii* fortified jaggery**

Helminthiasis is the most mistreated tropical disease and imposed a great risk on human health and economy of the world. Poor sanitation, unsafe water, poverty, malnutrition and unawareness in the society have favored its propagation in humans and livestock (Brooker *et al.*, 2006). Helminth or worm infections have endured moderately high over time; women and children are more susceptible for helminthiasis (Jinabhai *et al.*, 2001). Normally, helminth infection do not cause noticeable ailment in humans. However, helminth's metabolic activities and toxic excretory products affect host's health integrity (Deacon, 2006).

Further, helminthiasis influences host's body for higher vulnerability to bacterial and other opportunistic infections (Bentwich *et al.*, 1995; Brooker *et al.*, 2004). The antihelmintic drugs are expensive, often out of reach for the public. Most of helminthes have established rapid resistance for these drugs (Melo *et al.*, 2003). In traditional system of medicine, a number of medicinal plants are used for the treatment of helminth infection since decades (Waller *et al.*, 2001). There is a great potential for discovery and development of effective drug from natural plant source against gastrointestinal parasites. *In vitro* antihelmintic activity of *D. hamiltonii* fortified jaggery of three-tested cane varieties studied using earthworms and the results revealed that no statistically significant difference both in paralytic and death time of helminthiasis between control and fortified jaggery of all sugarcane varieties as tabulated in Table 3. Control and *D. hamiltonii* fortified jaggery observed for uniform antihelmintic activity. The observed death time of worm was between 18-20 min at 0.5g/mL jaggery concentration. The killing time of earthworms by the standard drug piperazine hexahydrate

was 26.58 min at 10 mg/mL concentration. However, distilled water did not show any effect on life of helminthes and served as negative control. Further, jaggery concentration to bring about helminthic paralysis was approximately 50 folds higher compared to standard drug piperazine hexahydrate. Flaccid paralysis of the worm was the predominant effect of piperazine hexahydrate that resulted in expulsion of the worm by peristalsis. Jaggery prepared from sugarcane juice and palm sap sources demonstrated antihelmintic activity (Prasad *et al.*, 2010). Further, jaggery has acidic pH and contains mixture of sugars that results in low water activity.

The acidic pH and saturated sugars makes unfavorable condition for growth of helminthes in gastrointestinal tract. In addition, polyphenolic compounds are known to exhibit antihelmintic activity (Bate-Smith, 1962). Literature revealed that tannins displayed antihelmintic activity (Athnasiadou *et al.*, 2001). Phenolic compounds found to uncouple oxidative phosphorylation and halt respiration and thus mediate death of helminth parasites (Martin, 1997). The results revealed that the enhanced phenolic components in jaggery upon fortification with *D. hamiltonii* are not enough to bring down the death time of helminthic parasites.

In conclusion the fortification of sugarcane jaggery with *D. hamiltonii* root extract imparts good coloration to jaggery in addition to increased turbidity, insoluble solid contents and lower filterability. Further, no change in key chemical constituents like sucrose, and reducing sugar content was evident. However, enhanced total protein, phenolics and flavonoid content was observed. The enhanced phenolics and flavonoid content might be responsible for the exhibited higher DPPH radical scavenging, reducing power and lipoxigenase inhibitory activity. Hence,

D. hamiltonii fortified jaggery of sugarcane varieties Co 86032, Co 419 and Co 62175 might be used as a nutraceutical sweetener for its observed health benefits.

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Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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