Bioactive properties of *Caryota urens* L. (Kithul) sap and treacle and isolation and molecular characterization of economically useful yeasts from fermenting sap

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Abstract

Caryota urens L. is an underutilized palm that grows naturally in Asian countries including Sri Lanka. Unlike in most of the other countries, in Sri Lanka this palm has been traditionally used to manufacture treacle and jaggey, which have been considered as healthy natural sweeteners with antidiabetic claims from very ancient time. At present, with increased prevalence of diet related non-communicable diseases in the world, the value of these functional natural sweeteners are remarkably high. Therefore, in this study, bioactivities of *C. urens* treacle, related to traditionally claim antidiabetic property which included antioxidant, antihyperglycemic and lipid lowering were assessed. *Caryota urens* treacle is a time tested traditional sweetener, but scientific evidence for its toxicological safety is lacking. As such, toxicology of *C. urens* treacle was also evaluated. Yeast fermentation is reported to enhance bioactivities of beverages such as grape wine but its effect on bioactivities palm saps is not reported. In this study an attempt was made to isolate and molecular characterize wild yeast strains, from *C. urens* phloem sap which are having potential industrial application in production of bioactive beverages.

Antioxidant, antihyperglycemic and lipid lowering activities of *C. urens* sap and treacle were studied using *in vitro* and *in vivo* bioassays. *In vitro* antioxidant activities of sap and treacle were determined using radical scavenging, ferrous ion reducing power, oxygen radical absorption capacity and metal ion chelating assays using 96-well microplates. Further, ascorbic acid, phenolic and flavonoid contents were also determined. α-Glucosidase and α-amylase inhibition assays were used to evaluate *in vitro* antihyperglycemic activity of the sap and treacle. Rat models were used to study *in vivo* antioxidant, antihyperglycemic, lipid lowering activities and toxicological evaluation of the treacle. Data collected in all *in vivo* and *in vitro* experiments were statistically analyzed using Statistical Analysis Software (SAS) v 6.21. Yeast strains which showed economic potentials; either enhancement of antioxidant activity of the sap during fermentation or production of carotenoid pigments were isolated and characterized using molecular biology techniques. Polymorphism in internal transcribe spacer region1, 5.8S ribosomal gene and internal transcribe spacer region 2 (ITS1-5.8S-ITS2) of ribosomal gene cluster of yeasts and nucleotide sequence alignments of the gene were used in molecular characterization.

Caryota urens sap and treacle showed 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity (0.82 ± 0.29 and 0.15 ± 0.90 mmole TE/100g), 2,2-azinobis-3-

ethylbenzothiozoline-6-sulfonic (ABTS) radical scavenging activity (1.43 ± 0.21 and 0.58 ± 0.07 mmole TE/100g), ferric reducing power (FRAP) activity (2.56 ± 0.35 and 2.65±0.22 mmole TE/100 g), oxygen radical absorption capacity (2.09 ± 0.12 and 3.71 ± 0.19 mmole TE/100g) and metal ion chelating capacity (IC_{50}: 4.37 ± 1.44 and 19.60 ± 3.90 mg/ml). When the treacle was added to rat diet (60 % (w/w)) and fed for 28 days antioxidant activity o f rat serum increased by 15 % compared to control diet. *Caryota urens* treacle exhibited markedly low (46 %) postprandial glycemic response at fasted and fed states in rats when orally administered at 2.75 g/ kg body weight dose which is equitant to 20 ml/serving/normal adult man. In addition, it showed marked reduction in serum triglycerides (21 %), low density lipoprotines (32.5 %) and increase in high density lipoproteins (56.5 %). The results of safety evaluation showed that the treacle was well tolerated (in terms of food intake, growth, overt sings and aversive behaviors) and was neither hepatotoxic (in terms of serum alanine aminotransferase and glutamic oxaloacetic transaminase) and renotoxic (in terms of serum proteins, creatinine and urea) nor hematotoxic (in terms of standard parameters including blood cell and platelet counts).

In this study, 11 yeast isolates showed fermentation of sap resulting 4 - 6 % (v/v) ethanol and increase in antioxidant activity at room temperature (25 ± 2 °C). Further, when the sap is fermented at 8 ± 2 °C, 7 yeast strains produced less than 0.7 % (v/v) ethanol while increasing antioxidant activity by 4 folds indicating potential in development of low or non-alchoholic bioactive beverages. In molecular characterization, all these yeast strains along with a type strain of *Saccharomyces cerevisiae* produced 880 pb size PCR product of ITS1-5.8S-ITS2 which further produced similar banding pattern when separately digested with *Cfo I, Dde I* and *Hin f I.* In addition, all pigmented yeast isolates (7) along with a type strain of *Rhodotorula mucilaginosa* produced 640 bp size PCR products and similar banding pattern when separately digested with *Cfo I, Hea III* and *Hin fI.* In confirmation, nucleotide sequences of fermenting and pigmented yeasts were aligned with *S. cerevisiae* (ATCC 208606) and *R. mucilaginosa* (Y 17497) sequences in Genbank respectively. *R. mucilagenosa* when mass cultured in low cost culture broth produced 26.8 mg of pigment/ g of dry yeast. The crude pigment was preliminary identified as mixture of carotene, troluine and troluhodine.

In conclusion, this study showed multiple bioactive properties including antioxidant, antihyperglycemic and lipid lowering in a single natural sweetener, *C. urens* treacle. Further, findings of this study provide scientific justifications for the traditional health claims such as antidiabetic. In addition, even at very high dose, safety profile of the treacle was remarkable. *C. urens* sap possesses comparable *in vitro* bioactive efficacy and phyto-constituents,

irrespective of the location of palm tapping. On the other hand, this study, for the first time, reports isolation and molecular characterization of *S. cerevisiae* strains that undergo fermentation at very low temperature such as 8 °C while enhancing antioxidant activity and *R. mucilagenosa* strains that produce carotenoid pigments from *C. urens* sap. Outcomes of this study will add value to *C. urens* sap and treacle and uplift the traditional *C. urens* industry in Sri Lanka. Further, bioactive potentials of *C. urens* sap and treacle demonstrated in this study will induce more studies in evaluating functional values of other palm saps and treacles in the world.