RESEARCH ARTICLE
ANTIOXIDANT ACTIVITY OF SUGARCANE JAGGERY WITH NEEM (AZADIRACHTA INDICA) LEAF EXTRACT

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ABSTRACT

Sugarcane jaggery fortified with Neem (Azadirachta indica) leaf extract at different concentrations were evaluated for antioxidant ability by DPPH assay, superoxide radical scavenging assay and reducing power assay. Quenching ability of neem-fortified jaggery for free radical DPPH and superoxide radical was decreased and increased with dose dependency neem fortification, respectively. An EC50 of 6.49, 5.0, 4.46, 4.10, 3.84 and 3.37 mg/mL was observed in Jag Control, Jag 1.3, Jag 2.6, Jag 3.9, Jag 5.2 and Jag 6.5% neem fortified jaggery for superoxide radical scavenging ability. Neem fortified jaggery indicated increased reducing power potential in dose dependent manner. Jag 6.5% neem fortified jaggery showed highest reducing ability than Jag Control for 0.5 to 2.5 mg/mL concentration. Data suggested the potential ability of neem-fortified jaggery to exhibit antioxidant activity.

MATERIALS AND METHODS

Preparation of Jaggery

Neem fortified jaggery prepared following the method described by JagannadhaRoaPVK, Madhusweta Das, Das SK2007. Briefly, neem leaf powder at different concentrations (1.3, 2.6, 3.9, 5.2 and 6.5% w/v) was added to Sugarcane juice extracted and pH was adjusted to 6.6 using Milk of Lime [Ca(OH)2]. The juice initially boiled for 10 minutes and the scum formed during boiling completely removed through filtration using muslin cloth. Finally, the juice was heated and concentrated to thick syrup until the temperature reaches 118 °C. The syrup cooled and transferred to chocolate moulds to obtain desired shapes. Jaggery prepared without the addition of neem served as control.

Total Phenol Determination

The total phenol content of jaggery determined spectrophotometrically using Folin-Ciocalteu’s method (Singleton VL, Orthofer R, Lamuela-Raventos RM1999). A sample aliquot of 100 µL (5%) added to 900 µL of water, 1 mL of Folin-Ciocalteu reagent (1:2, v/v) and 2 mL of 10% sodium carbonate sequentially, mixed thoroughly and incubated for one hour at room temperature. The absorbance measured at 765 nm in visible spectrophotometer. Gallic acid used as standard and the total phenolic content expressed as milligrams of gallic acid equivalent (GAE) per gram sample.

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of jaggery evaluated as per the method described by Yamaguchi T et al.,(1998). An aliquot (10-50 µL) of 5% jaggery samples and a standard antioxidant (BHT) of various concentrations made up to 200 µL using distilled water and then mixed with 1 mL of 0.1mM DPPH in

INTRODUCTION

Jaggery, a sugar rich food product is produced and consumed worldwide under different names such as Gur/Desi (Pakistan), Kokuto (Japan), Panela (Mexico, Panama and South America), Rapadura (Brazil), Hakura (Srilanka) and so on (Walter RJ 2012). It is prepared traditionally by concentrating sugarcane (Saccharum officinarum) juice. Sugarcane is world’s largest commercial crop cultivated extensively for its sucrose content and ethanol production. Sugarcane contains various phenolic compounds and its extracts displayed a wide range of biological activities including antioxidant, anti-inflammatory, anti-thrombosis, immunostimulation and anti-stress effects (El-Abasy M et al., 2002). Jaggery has great nutritive and medicinal value. Indian Ayurvedic medicine considers jaggery as medicinal sugar for treating throat and lung infections. In vivo studies dietary supplement of jaggery to exhibit health benefits. Dietary jaggery reduced the development of atherosclerosis (Okabe et al., 2009), lowered the incidence of chromosomal aberration due to arsenic toxicity (Nrusshanth S et al., 2008) and exhibited protective effect against lung damage induced by coal, silica dust and other particulate matter (Sahu AP, Saxena AK 1994). Jaggery being at least processed sugar retains phenolics and other phytochemicals of sugarcane and thereby finds to exhibit biological activities like antioxidant, cytoprotective and anthelmintic activity as reported in literature (Harish Nayaka MA et al., 2009; Prasad P et al., 2010). There are few reports available on jaggery fortification with vitamin C- Indian gooseberry (Anwaar et al., 2009). Mixture of Jaggery and Neem are consumed in traditional South Indian festival UGADI that symbolizing good and bad in life. Neem leaf is known to exhibit wide range of health benefits (Duke, James A 1992). The present investigation aims to evaluate neem fortified jaggery for antioxidant potential.

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methanol. The mixture was shaken vigorously and allowed to stand for 20 min in the dark at room temperature. The absorbance of the resulting solution read against control at 517 nm using a spectrophotometer. The ability to scavenge DPPH radical calculated using the following equation.

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

Superoxide Radical Scavenging Assay

The superoxide radical scavenging assay of jaggery was determined according to the method described by Nishikimi M, Rao NA, Yagi K 1972). Briefly, an aliquot (10-50 µL) of 5% sample and standard antioxidant Trolox mixed with 1 mL of Nitroblue tetrazolium, NBT (156 µM) and 1 ml of NADH (468 µM), Reaction was initiated by addition of 100 µL of Phenazinemethosulphate (60 µM) and the reaction mixture was incubated at room temperature for 5 min. The absorbance was read at 560 nm against blank spectrophotometrically and percentage of inhibition was calculated.

Reducing power Assay

The reducing power of jaggery was determined according to the method reported earlier (Yen GC, Chen HY 1995). Different concentrations of jaggery (0.5- 2.5 mg/mL) or standard antioxidant Trolox, (10-50 µg/mL) mixed with an equal volume of 0.2 M Phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture incubated at 50 °C for 20 min. An equal volume of 10% trichloroacetic acid added to the mixture and centrifuged at 3000 g for 10 min. The upper layer of the solution mixed with distilled water and 0.1 % FeCl₃ at a ratio of 1:1:2 (v/v/v) and the absorbance measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

All the experiments were carried out in triplicates (n = 3) and the results expressed as mean ± standard deviation (SD) using Microsoft Excel software.

RESULTS AND DISCUSSION

Total Phenol Content

Total phenol content of neem fortified jaggery has increased in a dose dependent manner and it is estimated that Jag Control, Jag 1.3, Jag 2.6, Jag 3.9, Jag 5.2 and Jag 6.5% contains 2.88, 2.96, 3.20, 3.50, 3.69 and 4.04 mg GAE/g, respectively as shown in Fig 1. Phenolic compounds in neem leaf extract has enhanced total phenol content in fortified jaggery.

Antioxidant Assays

DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical characterized with typical deep purple color and has maximum absorbance at 517 nm. It is widely used for evaluation of free radical scavenging effectiveness of various antioxidants. As shown in Fig 2, Antioxidant activity of neem fortified jaggery of varying percent evaluated by DPPH assay showed to decrease with increase in neem concentration. Neem fortified jaggery and Standard BHT exhibited free radical scavenging activity with EC_{50} of 862, 915, 1102, 1172, 1265, 1530 mg/mL and 6.75 µg/mL for ag Control, Jag 1.3, Jag 2.6, Jag 3.9, Jag 5.2 and Jag 6.5% and BHT, respectively.

Superoxide radical (O_{2}^-) called as hyperoxide are ROS generated during normal physiological process mainly in mitochondria during cellular respiration. Although superoxide radicals are weak oxidants but they give rise to powerful and dangerous hydroxyl radicals as well as singlet oxygen which induce oxidative damage for biomolecules. Fig 3 shows increased superoxide radical scavenging ability of neem fortified jaggery. Jaggery control and its fortified samples found to scavenge superoxide radical in a concentration dependent manner with EC_{50} of 6.49, 5.0, 4.46, 4.10, 3.84 and 3.37 mg/mL was observed for Jag Control, Jag 1.3, Jag 2.6, Jag 3.9, Jag 5.2 and Jag 6.5%, respectively An EC50 0.28 mg/mL for standard trolox was observed Jag 6.5% neem concentration jaggery was found to be potent superoxide radical quencher.

Reducing power is one mechanism of action of antioxidants and may serve as significant indicator of potential antioxidant activity. Fig 4 shows the reducing activity of neem fortified jaggery and is based on reduction of Fe^{3+}/ferricyanide complex to Fe^{2+} ferrous
complex monitored by measuring the formation of perl’s Prussian blue at 700nm. Reducing potential of neem fortified jaggery indicated increased reducing power ability in dose dependent manner compared to control jaggery. Jag 6.5% sample showed highest reducing power potential.

CONCLUSION

Jaggery fortified with neem leaf extract has the ability to quench oxidative radicals and thereby reducing oxidized stress. Hence neem fortified jaggery exhibits antioxidant potential and may by blocking the oxidizing chain reactions and thus the consumption of neem fortified jaggery may have enhanced health benefits

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