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Antioxidant Activity and Total Polyphenolic Content in the Fruit Juice of *Syzygium cumini* (L.) Found in the Dibrugarh District of Assam

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ABSTRACT

Fruit contains antioxidant property showing various health beneficial effects. The study was conducted to determine the antioxidant potential and total phenolics and flavanoid contents of Syzygium *cumini* (L.) fruit juice. The IC_{50} value of DPPH scavenging assay of fruit juice was 1.08±0.005 (mMT/L±SD) and the IC_{50} value of ABTS estimation was 0.49±0.03 (mMT/L±SD) expressed as trolox equivalents in mMT/L. The total flavanoid content (TFC) value was 34.38±0.81 (µg/ml±SD) expressed as quercetin equivalents (QE) in microgram per millilitre (µg/ml) and total phenolic content (TPC) value as 20.63±0.22 (µg/ml±SD) expressed as gallic acid equivalents (GAE) in microgram per millilitre (µg /ml).

Key words : *Syzygium cumini* (L.), traditional, antioxidant, trolox equivalents

INTRODUCTION

Fruits possess several phenolic components such as phenolic acid, anthocyanins and flavanoids which show a good antioxidant property which helps in the protection of the cells from damage by free radicals. As these free radicals can damage the cell causing various coronary diseases, cancer and several other diseases. The natural antioxidants can reduce food deterioration by lipid peroxidation during storage and processing (Sahin and Demir, 2016).

Antioxidant rich fruits can block the damage of proteins, lipids and nuclide acid molecules from oxidative stress caused by non-radicals and reactive oxygen species (ROS). Antioxidants bind to the metal ions which quence superoxide and singlet oxygen and reduce hydrogen peroxides leading to the suppression of the free radicals (Silva and Sirasa, 2016).

Syzygium cumini (L.), also known as Indian blackberry, is found in various places of Asia such as India, China and Malayasia. It belongs to the Myrtaceae family. *S. cumini* has huge application in various India traditional systems of medicines; unani, ayurveda and siddha. Medicinal property of various kinds has been reported in *S. cumini* (L.) as antimicrobial, antidiabetic, anti-inflammatory and free radical scavenging activity (Priya *et al.*, 2017). Fruits like Jambolan *S. cumini* (L.) is reported to have important antioxidant property and various pharmacological properties too which help in the reduction of oxidative stress that harms the body by causing several chronic degenerative diseases such as diabetis, hyperlipidaemic, diarrhea, allergy, arthritis, ulcer, etc. (Mehta *et al.*, 2017; De Morais Sousa *et al.*, 2021).

Historical reports indicate that before the discovery of insulin, Jambolan was used in the treatment of diabetes in India as well as in many other countries (Singh *et al.*, 2016). Therefore, the present study was undertaken to study the antioxidant and total phenolic content of the fruit juice of *S. cumini* (L.).

MATERIALS AND METHODS

Mature and ripen *S. cumini* (L.) fruits were purchased from local market of Chowkidingee, Dibrugarh district, Assam, India and brought to the laboratory. The fruits were washed in distilled water properly and dried for 1 h. The fruit juices samples were prepared by crushing followed by filtering the samples into the sieve containing layers of muslin cloth. The juice sample was collected and the residues were removed by centrifugation, and the filtrates were bottled to use for the various analytical tests.

Reducing sugar was estimated by the standard

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biochemical method using 3,5-dinitrosalicylic acid reagent and glucose (stock concentration of 1 mg/ml) as standard (James B. Sumner, 1921) describe by Ghosh *et al.,* (2016).

Antioxidant Activity by DPPH Free Radical Scavenging Assay

The DPPH method for antioxidant activity of the sample was assessed through the rate of decay in the absorbance at 517 nm (Padilha *et al.*, 2017). The DPPH radical solution (1 mM) was prepared in ethanol and diluted to an absorbance of 0.900±0.05. The absorbance of the DPPH solution was determined at time t=0 min and 30 min after the addition of sample. The absorbance of the DPPH control was also noted. The scavenging activity of the juices was calculated using the formula :

$$\text{Scavenging activity (\%)} = [(A - B) / A] \times 100$$

Where, A was absorbance of DPPH and B was absorbance of DPPH and sample combination.
The ABTS radical (1 mM) was formed through the reaction of 7 mM ABTS in 140 mM potassium persulfate in the absence of light for 16 h (Padilha *et al.*, 2017). The solution was then diluted in ethanol until an absorbance of 0.700±0.05 was obtained. The ABTS radical scavenging activity of the sample was determined through the rate of decay in the absorbance at 734 nm determined at time t = 0 min and at time t = 6 min after the addition of the sample.
The total phenolic content in the fresh fruit juice/fruit brew sample was measured according to the Folin – Ciocalteu's procedure (Singleton and Rossi Jr, 1965) described by (Seal and Chaudhuri, 2016). 20-100 µl of the tested samples were taken into test tubes. 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added to the tubes. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer, Systronics). All samples were measured in triplicate. The results were expressed in µg/ml of gallic acid equivalents (µg GAE/ml).
Total flavonoid content was determined according to the method (Aourabi *et al.,* 2018). One milliliter of sample was mixed with 1 ml of 2% $AlCl_3$ X $6H_20$ solution and incubated at room temperature for 10 min. Thereafter, absorbance was measured at 430 nm. Total flavonoid content was calculated as the quercetin equivalent in µg/ml of sample.
The graphs were plotted in Microsoft Excel 2010. The values were calculated for their mean and standard deviation (Mean±SD).

RESULTS AND DISCUSSION

The pH value of the fresh fruit juice was acidic which ranges from 5 to 6. Low pH value was helpful for prevention of the growth of microorganisms which were pathogenic to the fruit juice (Kim *et al.*, 2017). Total reducing sugar content of fresh *S. cumini* (L.) juice was 1.25± 0.02 (µg/ml)±SD.
DPPH free radical scavenging activity was used to determine the antioxidant ability of fresh fruit juice. Spectrophotometric analysis showed that DPPH assay was 1.08 ± 0.005 mMT/L±SD and ABTS was 0.49±0.03 mMT/L± SD) expressed as trolox equivalents in mMT/L (Table 1). Therefore, in DPPH assay of the *S. cumini* (L.) fruit juice showed greater antioxidant activity as compared to ABTS assay (Figs. 1, 2 and 3).

Table 1. Spectrophometric assay of DPPH, ABTS to determine the antioxidant activity of fruit juice sample

Sample	DPPH (IC_{50}) (mMT/L±SD)	ABTS (IC_{50}) (mMT/L±SD)
Fresh juice	1.08±0.005	0.49±0.03

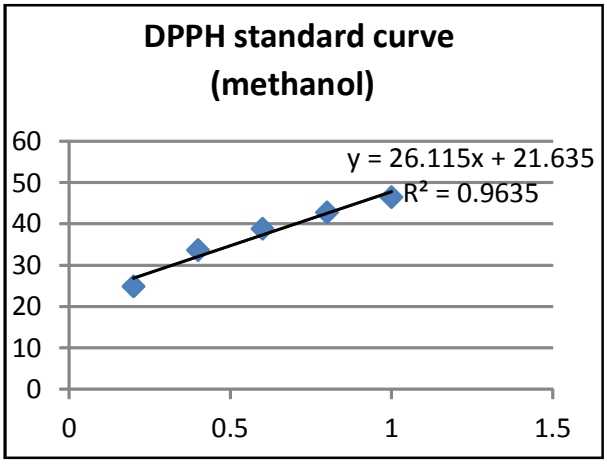

Fig. 1. Standard curve of DPPH assay by trolox.

DPPH was a free radical stable cation that scavenged the sample from purple to yellow at maximum absorbance range 517 nm using methanol as solvent. Thus, higher was the

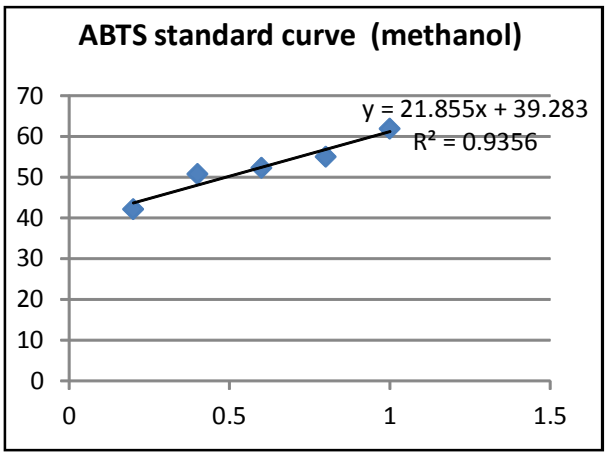

Fig. 2. Standard curve of ABTS assay by trolox.

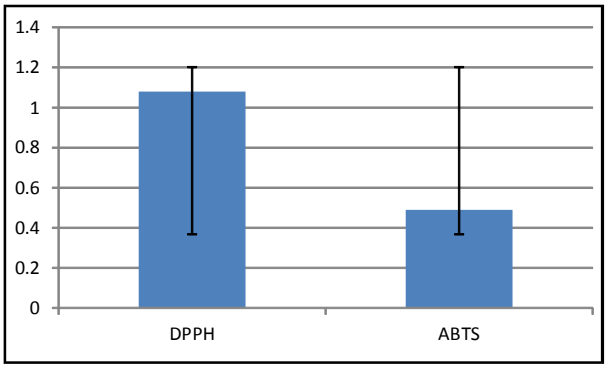

Fig. 3. Bar chart for DPPH vs. ABTS assay.

antioxidant property in the sample greater was the inhibition of the DPPH free radicals. However, ABTS also scavenged the free radicals cations at maximum absorbance range 734 nm. Oxidation of ABTS formed green coloured free radical. ABTS assay was highly useful in experimentation of coloured food products (Singh *et al.*, 2016).

The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per millilitre (µg /ml) of sample using the following equation based on the calibration curve y = 0.0158 x + 0.347; R^2 = 0.9868; where y was the absorbance and x was the gallic acid equivalent (µg /ml). The total phenolic content of the fruit juice sample was observed to be 20.63±0.22 (µg /ml±SD; Table 2; Fig. 4).

Table 2. Comparison of total phenolic content and total flavanoid content

Sample	TPC (µg/ml±SD)	TFC (µg/ml±SD)
Fresh juice	20.63±0.22	34.38±0.81

The total flavonoid content was expressed as quercetin equivalents (QE) in milligram per millilitre (µg /ml) of the sample using the

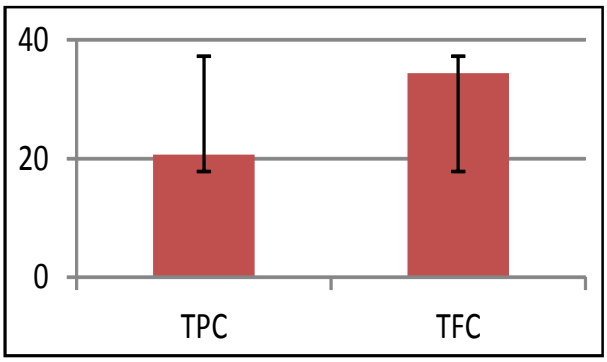

Fig. 4. Bar chart for comparision between total phenolic content and total flavanoid content.

following equation based on the calibration curve y = 0.0009x + 0.013, R2 = 0.9929 where y was the absorbance and x was the quercetin equivalent (µg/ml). The total flavonoid content of the fruit juice sample was found to be 34.38±0.81 µg/ml. Plant phenolics such as flavonoids and phenolic acids were the major contributors of antioxidant activity. Total flavanoid content level in fruit juice was positively correlated with the total phenolic content (Islam *et al.*, 2015). The secondary metabolite i. e. flavonoids possesses various health beneficial properties such as antidiabetic, antiaging, anticancer, anti-inflammatory, neuro-protective, anti-analgesic, antineurological, antimicrobial activities as well as for fibrocystic disease (Chhikara *et al.,* 2018).

CONCLUSION

Low pH and minimum reducing sugar were observed in the *S. cumini* (L.) fruit juice. In the DPPH assay, the *S. cumini* (L.) fruit juice showed greater antioxidant activity as compared to ABTS assay. The total flavanoid content was observed higher than total phenolic content. This revealed that total flavanoid content had higher contribution for the antioxidant activity than compared to total phenolic content. Therefore these findings also suggested that *S. cumini* (L.) fruit can be utilized as a valuable product in various pharmacologicals as well as beverage industries.

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