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Short Communication

ASSESSMENT OF ANTIOXIDANT ACTIVITY IN THE FRESH JUICE AND THE BREW FERMENTED FROM *Syzygium cumini* (L.) FRUIT

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Abstract

Background: *Several varieties of alcohol-based fermented products are used by ethnic communities of Assam. Notably, traditional alcoholic beverages used by these communities believed to contribute to the nutritive values of the food used in socio-cultural practices. Currently, researchers are giving more emphasis on the value addition of the locally available substrate using various traditional as well as modern technologies.* ***Objective:*** *The present study is an attempt to produce alcoholic beverages from S. cumini* (L.) *fruit by using a traditional starter culture and also to explore whether the fermentation of fruit juice leads to changes in the antioxidant properties of the S. cumini (L.) or not.* ***Materials and Methods:*** *Fresh S. cumini* (L.) *fruit was collected aseptically and the juice was extracted for further experiment. The antioxidant properties in fresh fruit juice and fruit brew were separately tested for antioxidant activity by using the DPPH method. The pH changes before and after fermentation were also recorded. In addition, the alcohol content of fruit brew was also determined by the chromic acid oxidation method.* ***Results and Discussion:*** *The fruit brew shows pH in the range between (5 – 6); alcohol content recorded up to a maximum of 3 %. The antioxidant activities increased significantly from* 54.35 ± 0.07 to 89.11 ± 0.07 *in fresh juice and from* 86.64 ± 0.36 to 95.75 ± 0.05 *in fruit brew (t*=0.038305) at P< 0.05. ***Conclusion:*** *The finding showed that fermentation process enhanced the antioxidant capacity against the free radicals in vitro than the fresh juice. Therefore, the product could be utilized as safer and beneficial functional beverages for consumers.*

Keywords: Kola Jamuk; Traditional starter culture; Antioxidant; Fruit brew.

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Introduction

Syzygium cumini (L.) Skeels belonging to the family Myrtaceae, known as Indian blackberry is one of the well-known species widely distributed in Asia (East India, Malaysia, and China) [1]. Traditionally, *S. cumini* (L.) is widely popular in India and it has been valued in Ayurveda, Unani, and Siddha [2]. The fruits and bark of *S. cumini* (L.) are reported to possess a variety of medicinal properties in the treatment of diabetes, ulcers, biliousness, dysentery, and its related complications [3]. So far, the major health benefits of *S. cumini* (L.) have been attributed to the secondary metabolites such as polyphenols which act as antioxidants [4]. Antioxidants of various substances possess the ability to protect the body from tissue damage caused by free radical due to the overproduction of reactive oxygen or nitrogen species (ROS/RNS) [5][6]. The ROS and RNS are the collective terms applied to free radicals that include superoxide, peroxyl, nitric oxide, nitrogen dioxide as well as those non-radical reactive intermediates such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) [7]. Living cells, which have a powerful scavenging mechanism to avoid excess ROS-induced cellular injury, become inefficient, with aging and external stress leading to metabolic distress. Hence, free radicals are implicated in various degenerative diseases such as arthritis, cancer, aging, heart diseases, AIDS, diabetes mellitus, a liver disorder, etc. [2, 3].

Notably, traditional alcoholic beverages prepared from fruit juices, though uncommon, have been used as a health drink in some ethnic communities of upper Assam which is believed to contribute to the nutritional values of various food habits. Fruit juices are fermented to produce fruit wine which enhances the flavor and nutritive value due to the liberation of amino acids and other molecules from the substrate by starter culture during the process of fermentation [8]. Fermentation also increases the shelf life and organoleptic qualities of the product. Health beneficial secondary metabolites can also be produced from less nutritive original substrates by the process of fermentation [9].

The present study was conducted to investigate the antioxidant activity and alcohol content in fresh fruit juice and the brew of fermented fruit.

Materials and methods

Collection of fruits

Mature and preferably ripe fruits of *S. cumini* (L.) were collected from a local orchard and brought to the laboratory under aseptic condition, cleaned and preserved for future use.

Preparation of fresh fruit juice sample

The fruits were crushed after mixing with distilled water (1:1) and divided into two parts. One part (X) was kept as a fresh juice sample for analysis and another part (Y) was sterilized at 120°C for 15 minutes in an autoclave and used for fermentation by the traditional starter culture. The fermentation process begins with the addition of the starter powder at the ration of 1:10 in sample Y and allowed to ferment for 4 to 5 days maintaining triplicates. Aliquots taken from the brew samples were filtered and used for the analysis of antioxidant properties. The alcohol content was determined in the distillate of brew.

Chemicals: All the chemicals used were of analytical grade.

Estimation of alcohol content

The alcohol content was determined by the chromic acid oxidation method following the association of official analytical chemists (AOAC 1990) [10]. At first, a given amount of fruit brew was distilled and the distillate was collected. The distillate was used to estimate the ethanol content in the brew sample.

In brief, the reaction mixture consists of a suitably diluted 1ml sample incubated with 4ml chromic acid reagent at 60 °C for 30 minutes, and thereafter the optical density was recorded at 600 nm using chromic acid with distilled water as blank. The percentage of alcohol in the beer samples was calculated by using Equation 1 and Equation 2.

$$\%\ \text{of ethanol in distillate} = \frac{\text{Total ethanolic content in the sample}}{\text{Sample or brew volume}} \times 100 \qquad \text{Eq. 1}$$

$$\text{Total ethanol} = \text{Sample OD} \times 0.0180 \times \text{number of dilution} \times \text{distillate volume} \qquad \text{Eq. 2}$$

Antioxidant activity by DPPH free radical scavenging assay

The antioxidant activity of samples was determined by estimating the scavenging effect of the sample on the DPPH as free radical [11]. The fruit juice (fresh) /fruit brew sample of different concentrations (10 µL to100 µL/mL) was prepared in distilled water. Now, 2 mL of the samples with different concentrations were applied separately to test tubes containing 2 mL of DPPH solution (0.002 % in methanol). The tubes containing the reaction mixture were incubated in dark for 30 minutes at room temperature and the optical density was measured at 517 nm using UV-Visible Spectrophotometer maintaining the DPPH solution as a control. The scavenging activity was calculated using Equation 3.

$$\% \text{ of Scavenging activity} = \frac{A-B}{A} \times 100 \quad \text{Eq. 3}$$

Where A is the absorbance of DPPH and B is the absorbance of DPPH plus sample.

Statistical analysis

All the data collected were analyzed by one-way ANOVA and differences among the mean of groups were calculated by Student's t-test. All the graphs were plotted in Microsoft Excel 2010. The values were calculated and expressed in Mean ± SD. The t-test was used to calculate the significance of difference at p-value for each group to measure the degree of change occurring in parameters observed.

Results and Discussion

The pH value in both the fresh fruit juice (pH=5.45) and fruit brew (pH=5.85) was in the acidic range. The alcohol content in the fruit brew was measured in the range from 0 – 3 %.

DPPH free radical scavenging activity was used to determine the antioxidant ability of fresh fruit juice and fruit brew in the present study. As the concentration of the fruit juice sample increases from (10 - 100 µl), the scavenging activity (%) successively increases from 54.35 ± 0.07 to 89.11 ± 0.07. Similarly, the fruit brew also showed increasing trends of scavenging capacity (%) from 86.64 ± 0.36 to 95.75 ± 0.05. The free radical scavenging activity was increased significantly (t-test of 0.038) after fermentation of the fruit juice at $p < 0.05$. For instance, Table 1 shows that there was a significant increase in % of DPPH scavenging level from 54.35 ± 0.07 in fresh juice to 86.64 ± 0.36 in the fermented brew at DPPH concentration of 10 µl. Similar studies have shown that the fermentation process facilitates the extraction of anthocyanins and other phenolic compounds from the pomace and by forming new polymerized pigments and polyphenols that shown to enhance the level of antioxidant activity [12]. Available literature suggests that mixed culture starters can induce an increase in antioxidant activity and polyphenol contents [13]. Thus, the fermentation process induces an increase in the antioxidant level of the fruit juice as compared to fresh juice with mild production of alcohol, which also supplements additional health benefits. However, further studies will be required to explain the exact biochemical mechanism involved in these phenomenon.

Table 1. DPPH scavenging activity (%) in fresh juice vs fruit brew

Fresh fruit juice (mean ± SD)	Fruit brew (mean ± SD)	t. test
54.35 ± 0.07	86.64 ± 0.36	
68.72 ± 0.03	89.31 ± 0.28	
84.76 ± 0.10	90.72 ± 0.07	0.0383
87.98 ± 0.16	92.77 ± 0.24	(Significant)
89.11 ± 0.07	95.75 ± 0.05	

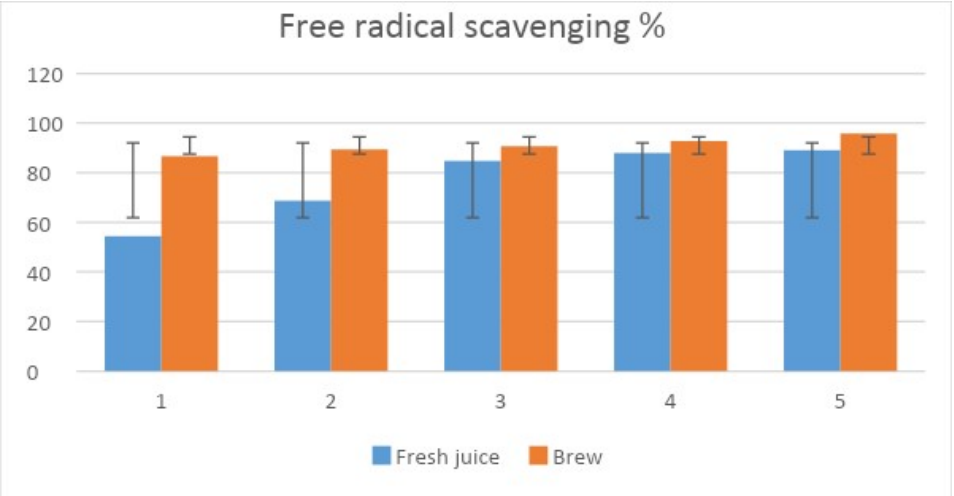

Fig 1: Free radical scavenging activity (%) mean ± SD of fresh fruit juice vs fruit brew sample.

Conclusion

The present study on the Indian blackberry indicated a bright prospect of fermentation technique for enhancing the nutritive value with a prolonged shelve life of the finished products derived from seasonal fruits readily available in the region. However, the present work is still in progress to analyze other phytochemicals and secondary metabolites in the fruit of *S. cumini* (L.).

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