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Sensory Assessment, Nutritional and Antibacterial Effect of Kombucha Incorporated with *Citrus grandis* (L.) Extract

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ABSTRACT

Background: Kombucha, a fermented tea beverage, has gained widespread popularity due to its potential health benefits. Traditionally produced by fermenting tea and sugar with a Symbiotic Culture of Bacteria and Yeast (SCOBY), kombucha offers antioxidant and probiotic properties. *Citrus grandis* (L.), an underutilized fruit from Assam, is known for its nutritional richness, but its use in fermented products is limited. This study aimed to develop a kombucha beverage incorporating extracts of *Citrus grandis* (L.) to enhance its nutritional profile and to explore its potential health benefits. The research further sought to evaluate the antioxidant activity, essential mineral content and safety of the developed formulations.

Methods: Five different kombucha formulations were prepared, including a control formulation, using varying concentrations of *Citrus grandis* extracts. The antioxidant activity was assessed through IC_{50} values and the vitamin C content was measured. Additionally, mineral composition, including essential elements such as iron, manganese, zinc and copper, was determined. Safety analysis included testing for the presence of heavy metals, specifically lead and cadmium.

Result: The *Citrus grandis* kombucha formulations exhibited significant antioxidant properties, with an IC_{50} value of 42.16, along with a high vitamin C content. The presence of essential minerals further enhanced the health benefits of the beverage. Overall, kombucha made from *Citrus grandis* demonstrates potential as a commercially viable product, combining traditional benefits with the unique properties of the fruit to create a nutritious, appealing beverage.

Key words: Antibacterial, Antioxidant, *Citrus grandis* (L), Kombucha, Scoby, Value addition.

INTRODUCTION

Kombucha is a fermented tea beverage made from a symbiotic culture of bacteria and yeast known as SCOBY and it has a high concentration of bio active chemicals generated from plant material (tea, juices, herb extracts) as well as microbial metabolic activity (Kapp and Summer 2019; Antolak *et al.,* 2021). The tea fungus originated in East Asia and was introduced to Europe from eastern Siberia, also known by other names, such as Chinese and Japanese mushroom. During the fermentation process of kombucha, several metabolites are created, which are responsible for its beneficial effects (Jakubczyk *et al.,* 2020). It is a symbiotic culture of bacteria and yeast that ferments regular tea for around two weeks to generate a high-end fruity workout beverage. In addition to being a low-sugar, pro-biotic-rich drink, kombucha provides the advantages of the tea used in its preparation, including its high antioxidant content (Hsieh *et al*., 2021). After fermentation, kombucha contains sugars, tea polyphenols, organic acids, ethanol, amino acids, vitamins (C and B), minerals and hydrolytic enzymes (Kapp and Summer, 2019; Laavanya *et al.,* 2021).

Citrus grandis (L.) or pomelo, is an underutilized fruit in Assam with significant potential for enhancing livelihoods and nutritional security (Sharma *et al.,* 2004). Despite its nutritional and economic benefits, pomelo is rarely cultivated. Studies emphasize its potential to improve food security and offer economic advantages (Barua *et al.,* 2019; Kumar *et al.,* 2019). Pomelo's antioxidant properties make it suitable for health supplements and functional foods. The increasing health consciousness among people has significantly altered consumption habits, leading to a surge in demand for fruit-based beverages in the market. (Pavithra and Mini, 2023). Blending of seasonal fruits and flowers in the form of food products can be a cost-effective way to fulfill everyday nutritional requirements (Singh and Sharma, 2019 and Sahrawat and Chaturvedi, 2022).

It is evident that Kombucha tea beverages will be a good alternative to carbonated beverages due to their therapeutic and health benefits. The product can easily replace many

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expensive and artificial antibacterial additives used in the food industry. It can also be an alternative to the use of artificial or synthetic additives in food production because in recent years this is becoming a major issue and problem for the concern for the consumers. Therefore, the present research was taken up to investigate the sensory attributes, nutritional composition, total phenol and flavonoid content, antioxidant and antibacterial activity and vitamin C content of kombucha incorporated with *Citrus grandis* (L.) extract.

MATERIALS AND METHODS

Preparation of kombucha

In the present study, raw materials for the preparation of kombucha tea were collected in January 2024. Tea leaves, sugar and fresh *Citrus grandis* fruit were procured from the local market of Guwahati and SCOBY (symbiotic culture of bacteria and yeast), which was ordered from Peepal Farm Products online. The whole experiment was conducted within a period of 5 months. The value-added kombucha was prepared using a previously described method with slight modification (Zubaidah *et al.,* 2018 and Soto *et al.,* 2019). Plain kombucha was prepared in 1000 ml of water which was boiled at 100°C for 5-6 min. Sugar (100 g) and 3 teaspoon of tea leaves (three teaspoons) were added to water. After preparation of the tea, it was maintained at room temperature. The prepared tea was poured into a glass jar and SCOBY was added to the starter tea was mixed together. It was further fermented for 2 weeks at room temperature. For the *Citrus grandis* juice, 700 ml of fruit juice was extracted from approximately four medium-sized grapefruits. Sugar (300 g) was added to the juice to turn it into concentrated syrup and refrigerated in the jar until further use. After fermentation of plain kombucha for 2 weeks, SCOBY was removed and plain kombucha and *Citrus grandis* (L) were infused together and refrigerated for 1 day.

Formulation of kombucha treatments

A total of five formulations were developed in different variations, with a control formulation coded as C, containing 100% Raw or Plain Kombucha. The ratio of plain kombucha and *Citrus grandis* (L) extract for Treatment 1 (T1) was 80:20, for Treatment 2 (T2) was 70:30, for Treatment 3 (T3) was 60:40 and for Treatment 4 (T4) was 50:50 (Table 1). After mixing with fruit extract, the treatments were left to ferment for one more day so that both the flavors mixed well together and then served for sensory evaluation (Fig 1).

Sensory evaluation of the formulated *Citrus grandis* (L) kombucha

In the present study, a score card was made consisting of a table utilizing the hedonic ratings on 9 points scale from like extremely to dislike extremely. Sensory evaluation of the kombucha was performed by 35 semi- trained panelists. The most sensory-acceptable treatment of *Citrus grandis* (L) kombucha was chosen for nutritional analysis and shelf-life investigations at ambient and refrigerated temperatures.

Determination of physical parameters

Physical parameters, such as total soluble solids (TSS) and pH, were determined using a hand refractometer

Table 1: Formulation of various *Citrus grandis (L)* treatments.

Sample code	Plain kombucha	*Citrus grandis* (L.) extracts
Control (C) (100%)	500 ml	-
Treatment 1 (T1) (80% / 20%)	400 ml	100 ml
Treatment 2 (T2) (70% / 30%)	350 ml	150 ml
Treatment 3 (T3) (60% / 40%)	300 ml	250 ml
Treatment 4 (T4) (50% / 50%)	250 ml	250 ml

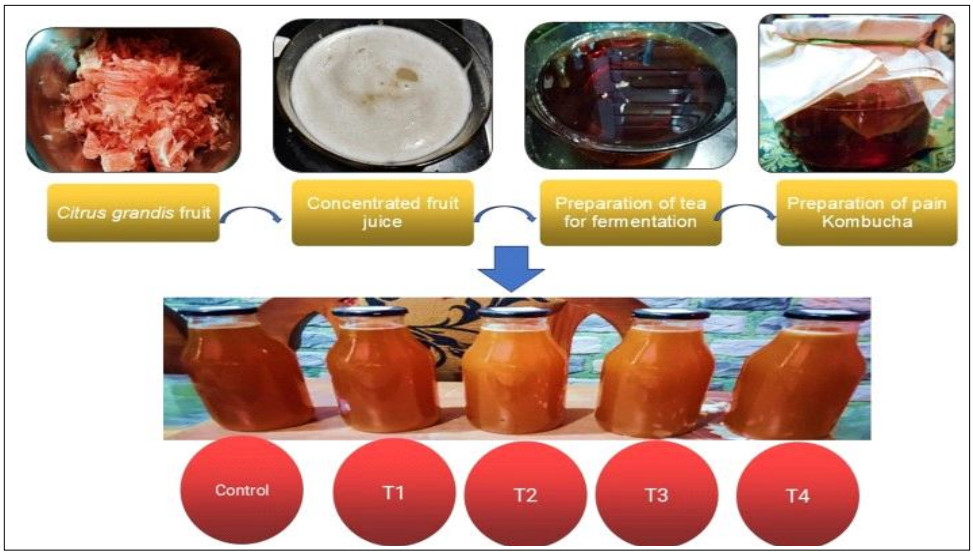


Fig 1: Processing of kombucha incorporated with various concentration of *Citrus Grandis* (L) extract where control was 100% of plain kombucha followed by T1-80:20, T2- 70:30, T3- 60:40 and T4- 50:50.

(ERMA) and digital pH meter (SYSTRONICS). To determine the pH of the developed product, a pH meter was used and the reducing sugar content was determined by the Lane-Eynon Titration Method (AOAC method) using standard procedures (Sewwandi, 2020).

Determination of nutrient content

Proximate analysis for total fat, protein and carbohydrate content was performed using AOAC (2005) methods. Mineral content (iron, manganese, copper, *etc*.) was determined by Atomic Absorption Spectrophotometer (SpectrAA 220, Varian, USA).Antioxidant activity was assessed using the DPPH method, calculating % inhibition with the formula:

$$\% \text{ scavenging of DPPH} = \frac{(A_{control} - A_{extracts})}{(A_{control})} \times 100$$

IC_{50} values were derived from a sigmoid curve using nonlinear regression.

Total phenolic content (TPC) was determined via the Folin-Ciocalteu method at 765 nm, while total flavonoid content was measured colorimetrically at 510 nm. Vitamin C was analyzed through HPLC (Agilent 1220 Infinity II) using a C18 column and a methanol-water mobile phase (30:70), with readings taken at 280 nm after sample filtration. This approach ensures accurate nutrient and bioactive compound analysis.

Antibacterial activity of the developed sample against *Escherichia coli*

Antibacterial activity was assessed using the broth microdilution method (Eloff, 1998) to determine the Minimum Inhibition Concentration (MIC). Overnight bacterial cultures were adjusted according to McFarland standards. Kombucha extracts were dissolved in acetone (10 mg/ml), with 100 µl added to the first well of a 96-well plate and serially diluted with water. *E. coli* dilutions were prepared and transferred to the plate. Antibiotics were added to the 11th row, while the 12th row served as a control. Azithromycin was used as a positive control and acetone was used as a solvent control. After it was kept in an incubation at 37°C for 24 hours, MIC was visually determined as the lowest concentration inhibiting bacterial growth. The shelf life of the product was studied and was done for 7 days by checking the microbial count in serial dilution method of the developed sample. Nutrient agar was used as a media to check the growth of the microorganisms.

Statistical analysis

All sensory evaluation data were statistically analyzed in triplicate. According to descriptive statistics, means were evaluated and are represented as mean±SD.

RESULTS AND DISCUSSION

Sensory evaluation of the developed kombucha

After sensory or organoleptic evaluation of the developed product, all the data were compared and analyzed using the Mean Acceptability Scores (Table 2). Among all five formulations, the most selected sample was T4, which contained equal amounts of Plain Kombucha (50%) and *Citrus grandis* (L) (50%) extract.

Physical parameters

As shown in Table 3, there was no significant change in the total soluble Solid from 1st day of fermentation (8.18) to

Table 2: Mean acceptability scores of the developed product.

Product code	Color (Mean±SD)	Appearance (Mean±SD)	Taste (Mean±SD)	Flavour (Mean±SD)	Mouth feel (Mean±SD)	Smell (Mean±SD)	Overall acceptability (Mean±SD)
Control	7.71±0.80	7.71±0.71	7.03±1.53	6.82±1.40	6.74±1.48	6.91±1.12	7.28±1.20
T1	7.54±0.91	7.6±0.81	6.77±1.26	6.82±1.09	6.82±1.01	7.2±0.93	7.11±0.96
T2	7.8±0.71	7.68±0.71	7.02±1.24	6.97±1.12	7.11±1.20	7.34±0.80	7.37±1.03
T3	7.68±0.90	7.48±0.81	6.74±1.29	6.8±1.34	6.77±1.33	7.02±1.17	7.39±1.13
T4	7.94±0.82	7.74±0.78	7.05±1.16	7.02±1.17	6.80±1.25	7.37±0.88	7.48±0.81

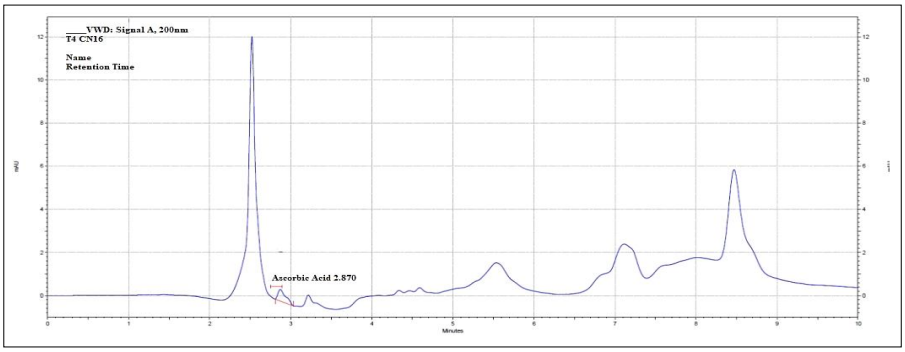


Fig 2: Chromatography of sample T4 at 280 nm and run time of 10 minutes.

10th day of fermentation (8.21). The Reducing sugar (Sugar as Sucrose) for the developed *Citrus grandis* (L) Kombucha was found to be 4.20 g /100 g on 1st day and 4.34 g / 100 g on 7th day. The pH of the developed *Citrus grandis* (L) kombucha was approximately 5.59, which was quite acidic on 10th day with a pH value of 3.35. The change in total soluble solids and reducing sugars in kombucha has been observed in similar studies (Wispen *et al.,* 2021). The pH of kombucha typically starts from 4.9 to 5.5 for black and green teas. As fermentation progressed, the pH dropped rapidly, reaching below 4.6 early on to prevent microbial contamination. By the end of primary fermentation, the pH usually falls between 2.5 to 3.5 for all fruit juice (Kitwetcharoen *et al.,* 2023).

Determination of nutrient composition

From Table 4 it was observed that the developed *Citrus grandis* (L) Kombucha had a fat content of 2.25 g/100 g, total protein content 0.04 g /100 g and the total carbohydrate 4.7 g /100 g. The iron, manganese and zinc contents of the developed product were 4.609 mg, 2.320 mg and 0.245 mg, respectively. The vitamin c content of the sample was 0.1049 µg/ml. DPPH scavenging activity was observed at 120 ug/ml in compared with that at different concentrations (74.33%) (Fig 3). The IC_{50} value of the developed sample was found to be 42.16 mg vit C/ml. The total phenol content and total flavonoid content assays recorded value of 83.40 mg/ml and 262.24 mg/ml respectively.

Present research is in consonance with a similar study in which researchers found significant levels of minerals such as calcium (1.5 to 4.0 mg), magnesium (1.0 to 2.5 mg), potassium (10 to 30 mg) and phosphorus (0.5 to 1.5 mg) per 100 Ml in different kombucha samples (Villarreal Soto *et al.,* 2018; Suhre *et al.,* 2021 and Içen *et al.,* 2023). Studies have reported that the vitamin C content in kombucha can increase significantly during fermentation, especially when enriched with ingredients such as jujube, where the vitamin C content could reach up to 0.00711 µg/100 mL (La Torre *et al.,* 2024). The antioxidant data aligns with those of other studies where the researchers revealed that the content of antioxidant compounds ranged between 70.62% and 94.61% DPPH scavenging activity for their kombucha samples (Jakubczyk *et al.,* 2020). The total phenol content (412.25 mg/l) was recorded for bamboo leaf kombucha fermented with leaf residues as recoded by Xiong *et al.* (2023). which is higher than the value recorded in the present study. This variation may be due to the effect of temperature and the processing method adopted.

Antibacterial activity of the developed sample against *Escherichia coli*

From the Fig 4 it was observed that the developed kombucha showed activity against the selected pathogen *i.e E coli* in

Table 3: Physical parameters of the developed sample.

Quality charactersitic	Results	
	1st day	10th day
Total soluble solid	8.18	8. 21
Reducing sugar	4.20 g/100 g	4.34 g/100 g
pH	3.59	3.59

Table 4: Nutritive analysis of the developed kombucha.

Parameters	Results
Fat (g/100 g)	2.25
Protein (g/100 g)	0.4
Carbohydrate (g/100 g)	4.7
Energy (g/100 g)	40.78
Iron (mg/100 mg)	4.609
Manganese (mg/100 mg)	2.320
Zinc (mg/100 mg)	0.245
Copper (mg/100 mg)	0.083
Total phenol content (mg/ml)	83.40
Total flavonoid content (mg/ml)	262.24

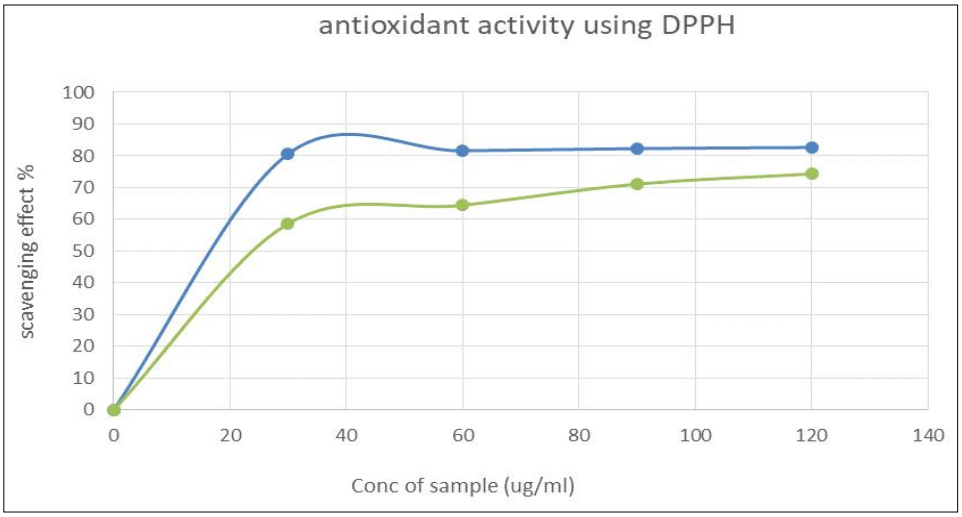


Fig 3: Graph representing antioxidant activity using DPPH, IC 50 value is 42.16±0.11.

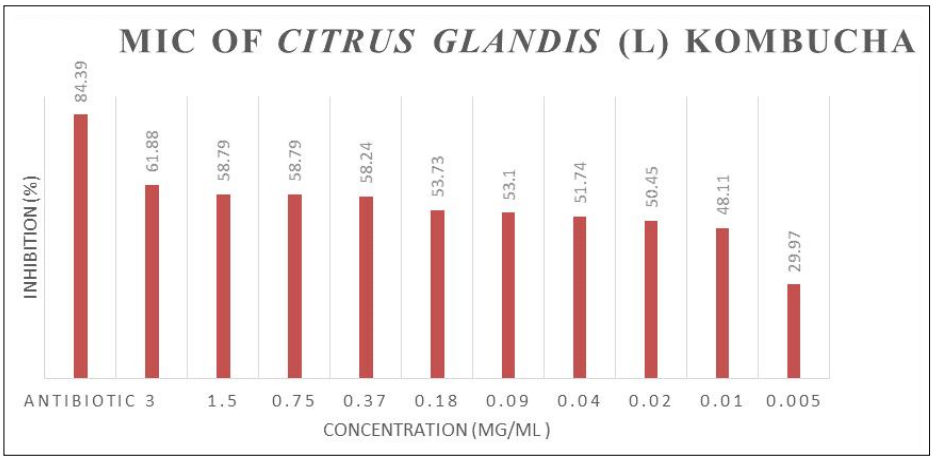


Fig 4: Graph representing the minimum inhibitory concentration of *Citrus grandis* (L) kombucha.

dose dependent manner which can be compared with standard antibiotic *i.e* Azithromycin. The lowest concentration, that is, less than 50%, also showed the minimum inhibitory concentration. Previous researchers have also reported strong antibacterial activity of kombucha against certain pathogens, such as *E. coli* and *Staphylococcus aureus* in their previous studies (Al-Mohammadi *et al.,* 2021).

From the microbial plate count method, it showed that after fermentation, the developed kombucha could be stored at room temperature for 7 days after which microbial growth was observed. In similar study for soursop kombucha researchers observed a decreased microbial growth after storing the kombucha at 4°C. It is also observed that prolonged storage in appropriate temperature may have high potential to improve the quality, metabolites content and biological activity of kombucha beverage (Tan *et al.,* 2020).

CONCLUSION

The present study was carried out to develop an underutilized fruit, *Citrus grandis* (L) of Assam, to make people aware of the nutritional and health benefits it possesses and to make this fruit available in their diet as well as in the market by transforming it into various forms of appealing food products. Selected and pre-processed fruit extracts of *Citrus grandis (L)* were used to develop a fermented drink known as kombucha. Therefore, this study aimed to develop low-cost, easily available nutritious food products incorporating *Citrus grandis* (L) that can be utilized to determine their organoleptic quality, bioactive properties and different methods and processes of value addition further processed to higher commercial levels. The popularity of kombucha is increasing because of its beneficial effects on human health, where the beverage is known to be rich in bioactive components that can be digested, metabolized and absorbed by the body. Also, it was thought that the use of different berry fruits as a raw material in the production of Kombucha tea will be more preferred by consumers in terms of flavor, taste and functional properties.

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Author's contribution

All authors significantly and directly contributed to data collection, data analysis, data presentation and discussion. The authors are responsible for the content and writing of this manuscript.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Ethics statement

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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