Rout *et al. Food, Nutrition and Health* (2026) 3:15
https://doi.org/10.1007/s44403-026-00058-z

ORIGINAL RESEARCH

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# Valorisation of *Buchanania lanzan* fruit extract through lactic acid bacteria submerged fermentation for lacto-juice production

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## Abstract

*Buchanania (B.) lanzan* is an excellent multipurpose tree well known for its medicinal and therapeutic values in Indian folk medicine. All the parts of this plant are used for preparation of various traditional medicines. In the present study, fruit extracts of *B. lanzan* were used for the production of lacto-juice through fermentation using three *Lactobacillus* strains, such as *Lacticaseibacillus (L.) rhamnosus* (MTCC 1408), *Lactiplantibacillus (Lp.) plantarum* (MTCC 1407), *and Levilactobacillus (Lvb.) brevis* (MTCC 1750). The crude fruit juice was used to analyze the presence of various phytochemical compounds, along with total sugar, lactic acid, and pH content. The results of the phytochemical study showed the presence of tannins, saponins, steroids, glycosides, phytosterols, terpenoids, triterpenoids, leucoanthocyanins, phlobatannins, and phenolic compounds. After fermentation, the pH of the *Lp. plantarum* fermented *B. lanzan* fruit juice decreased from 5.18 ± 0.71 to 4.82 ± 0.55, indicating the occurrence of better fermentation as compared to *Lvb. brevis, and L. rhamnosus* fermented juice with pH 5.18 ± 0.71 to 4.86 ± 0.45 and 4.93 ± 0.45, respectively. The *Lp. plantarum* fermented fruit juice showed the highest lactic acid production of 16.78 ± 0.3 g/L after 120 h incubation whereas that of the *Lvb. brevis* and *L. rhamnsus* had lactic acid concentrations of 14.98 ± 0.11 g/L and 12.78 ± 0.45 g/L, respectively. The sugar concentration of the *Lp. plantarum* fermented juice decreased from 147.11 ± 0.37 g/L to 75.91 ± 0.40 g/L after 120 h of fermentation, indicating the utilization of sugar by *Lp. plantarum* for lactic acid production. The *Lp. plantarum* fermented juice showed antioxidant and antibacterial activity. The fermented sample was further characterized using Fourier transform infrared, gas chromatograph-mass spectrometry (GC-MS), and scan electron microscope (SEM) analyses. The lactic acid peak was observed in GC-MS analysis, and SEM analysis showed attachment of *Lp. plantarum* in the fermented sample. All these indicate that the fermentated lacto-juice of *B. lanzan* shows high potential in the development of probiotic-based beverages with a vast spectrum of health benefits and other biological activities.

**Keywords** Lacto-juice, Fermentation, Lactic acid, Therapeutic, Probiotic, Beverages

## Introduction

In recent years, fermented beverages produced through lactic acid bacteria (LAB) (probiotic) fermentation are very much in demand [1]. Probiotic products and functional food products have gained significance attention and popularity demand in the market worldwide due to their benefits for improving human health [2]. It is reported that probiotic-enriched functional food products have increased their demand up to 60-70% in the global market [2]. The most frequently used probiotic-enriched functional foods, such as cheese, whey, yogurt, and kefir, are based on milk and its derivatives [3]. These fermented foods were not suitable for consumers with galactosemia, high cholesterol intolerance, or allergic to milk protein [3]. Moreover, the fermented milk product causes a change in color, texture, viscosity, and acidity,

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which reduces the interest of the consumer [4]. In this context, non-dairy probiotic products derived from processed fruits and vegetables, fish, cereals, and soy products have been found to overcome these problems [3, 5].

The fruit sector probiotic products from fruit sector have advantages, as these products are nutritious and contain high sugar, which favors the growth of probiotics [3]. Therefore, many studies have been conducted on diverse fruit juice based probiotic beverages. The fruit juice-based probiotic beverages are rich in vitamins, minerals, antioxidants, and dietary fibers [3]. Besides, multiple researches have shown that probiotic bacteria can convert the phenolic substance present in fruits into useful metabolites [6].

Many fruits were explored by researchers to produce probiotic beverages; however, fewer reports are present on fruits consumed by tribals that are underutilized[7]. Among these underutilized fruit species, *Buchanania (B.) lanzan* Spreng (Charkoli) is a socio-economically important fruit-bearing plant, which has high medicinal importance and is found in tribal dominated states of Odisha, Bihar, Chhattisgarh, Andhra Pradesh, Madhya Pradesh, Jharkhand, Rajasthan, Uttar Pradesh, Gujarat, Maharashtra and Karnataka [8]. As an under-exploited fruit crop, it gives monetary reward to the tribal community and seems to be a boon for them [9]. Apart from India, this plant is also found in Bangladesh, Nepal, Myanmar, Thailand, Laos, Cambodia, Vietnam, and southern China. It is a medium-sized deciduous tree belong to the family *Anacardiaceae*. It has a straight trunk and dark grey, rough bark; leaves are simple, alternate, and broadly oblong with a leathery texture. This tree bears drupaceous green fruits that become reddish-purple after ripe [10, 11]. This tree is valued as a culinary spice and thickening agent in traditional dishes. Beyond the utilization of fruit as food, seeds, barks, leaves, and other parts of the plant are utilized in Ayurvedic, Unani, and Siddha medicine preparation for their therapeutic properties in treatment of various ailments [11]. In pharmacy *B. lanzan* tree for the treatment of various disorders, is also used as tonic, cardio tonic products [12]. The seed oil of *B. lanzan* is used for cooking, cosmetic preparation where as the timber and other plant parts are used by rural communities for many other purposes [13]. Various secondary metabolites found in this tree are useful for its medicinal properties. Knowledge of the chemical constituents of plants is desirable due to their usefulness for the synthesis of complex chemical substances. To date, available review literature indicates that research on *B. lanzan* has primarily addressed on its nutritional value, antioxidant potential, and therapeutic relevance, while no studies have reported so faron fermentation of *B. lanzan* fruits or its application development fermented beverages. In this contex, controlled lactic acid fermentation using suitable *Lactobacillus* sp. widely accepted strategy for improving bioavailability, functional properties, and shelf life of plant fermented products. The fermented juices can be an usefull beverage that has excellent sensory expression and numerous properties.

Therefore, the present study aims to evaluate the fermentation of *B. lanzan* fruit juice using a suitable LAB and to enhance its nutritional and medicinal value, along with sensory attributes for its application as a quality health drink with multiple benefits. In this regard, the fermented juice was evaluated for antimicrobial, antioxidant and analyses of scan electron microscopy (SEM), Fourier transform infrared (FTIR), and gas chromatography-mass spectrometry (GC–MS) to determine and ascertain its biological activity, structural characteristics, and chemical compositions.

## Materials and method

### Procurement and preservation of Buchanania lanzan fruit sample

Fresh *B. lanzan* fruits were obtained from the market near the forest region of Balangir, 20.7011° N, 83.4846° E, Odisha, India. The fruits were washed and cleaned using tap water and stored in a refrigerator at temperature of 4℃ for the experiments.

#### *Extraction of B. lanzan fruit juice*

The previously stored fruit samples were thawed for 2 h before the experiments. The *B. lanzan* fruits were blanched with hot water for 1 min at 70 ℃. Then the juice was pasteurized at 80 ℃ for 5 min [14]. Fruit pulp was obtained by removing the seeds and blended in a mixer grinder. Water was added to the blended pulp to extract the juice. The ratio of water to fruit pulp was 2:1, 1:1, and 1:2 (w/v). The mixer with a water-to-pulp ratio of 2:1 produced the highest juice yield, while mixtures with water-to-pulp ratios of 1:2 and 1:1 produced lessthe lowest juice yield. Thus, only the ratio of 2:1 was used for future fermentation experiments. The turbidity, viscosity, pH, color, and refractive index, were estimated using the extracted fruit juice.

#### *Phytochemical analysis*

Preliminary phytochemical tests were carried out on water extract using standard methods for identification or for the presence or absence of phytochemicals such as alkaloids, tannins, flavonoids, steroids, saponins, cardiac glycoside, glycoside, reducing sugar, phenolic

compounds, phytosterols, quinones, triterpenoids, terpenoids, phlobatannis, and leuconthocyanin, [15–18].

#### *Starter culture preparation*

*Lactiplantibacillus (Lp.) plantarum* (MTCC-1407), *Levilactobacillus (Lvb) brevis* (MTCC-1750), *Lacticaseibacillus (L.) rhamnosus* (MTCC-1408) were cultured and maintained on the De Man-Rogosa-Sharpe agar(MRS) agar (Himedia, GM369-500G, Maharashtra, India) inclines/slants at -20 °C in a refrigerator [14]. Good-quality Mahakali grapes were purchased from the local market in Bhubaneswar and washed under running tap water. Grape juice was extracted using a mixer-cum-grinder. The juice was filtered through cheesecloth, and its volume was measured. Then an equal volume of water was added to the filtered juice. The juice was autoclaved, inoculated with 24 h cultures of *L. rhamnosus, Lp. plantarum,* and *Lvb. brevis* and juices were incubated at 37 ℃ for 24 h [19].

### Fermentation of B. lanzan using starter culture

The extracted B. *lanzan* fruit juice was sterilized, and then inoculated with one of the starter cultures of *L. rhamnosus, Lp. plantarum,* and *Lvb. brevis.* Then, these inoculated fruit juices were incubated at 37 ℃ for 120 h for fermentation. The LA and sugar concentration of fermented fruit juice were calculated at 24 h intervals up to 120 h (Fig. 1).

#### *Biochemical analysis of the raw and fermented B. lanzan fruit juice*

Both the BLF juice and the fermented *B. lanzan* fruit (BLF) lacto-juice were used to estimate the drop in pH, total sugar, concentration of LA, antioxidants, and antimicrobial activity.

#### *Estimation of pH*

The pH of the fermented BLF juice at 24 h intervals up to 120 h was estimated using a pH meter (Digital pH meter, Type- 335, Systronics). The pH of the extracted fruit juice was measured and considered as a control sample [19].

#### *Estimation of protein*

The amount of protein content of fermented and non-fermented BLF juice was estimated using Lowry's method [20].

#### *Estimation of total sugar*

Total sugar contents of BLF juice were measured using the protocol given by Mahadevan and Sridhar [21]. The estimated sugar was expressed in g/L of the juice.

#### *Estimation of lactic acid*

The LA concentration of the non-fermented and fermented BLF juice was measured using the standard protocol described by Amerine and Ough [22].

### Antioxidant activity

Antioxidant contents like DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and DMPD (N, N-dimethyl-p-phenylenediamine) antioxidant activity of the fermented BLF juice were carried out as described in [23].

#### *DPPH free radical scavenging assay*

The DPPH scavenging activity of the fermented BLF juice was measured according to the procedure described by Brand-Williams et al. [24]. The extract (100 µl) was added to 1.9 ml of DPPH (0.1 mM) and mixed properly. Then the mixed solution was incubated for 10 min, and the

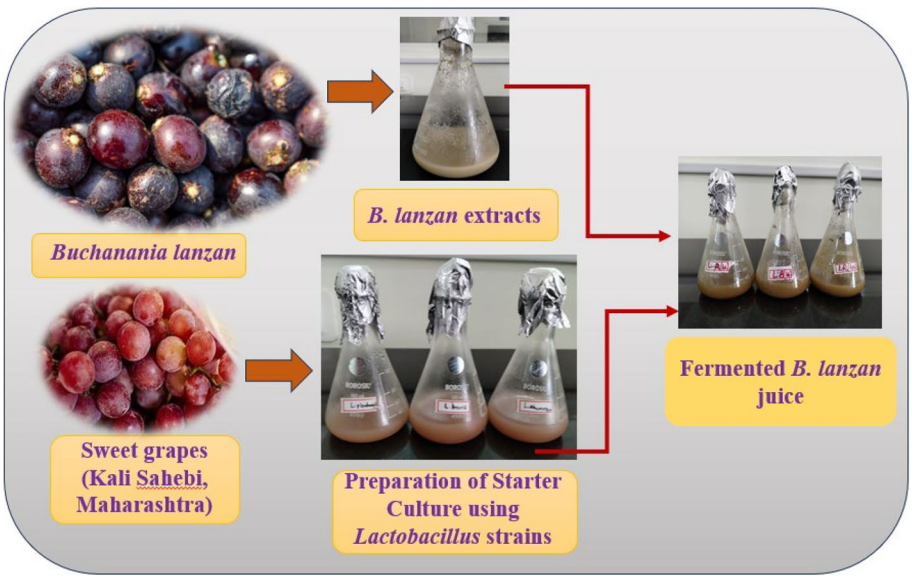



**Fig. 1** Methods for the Preparation of fermented *B. lanzan* lacto-juice

absorbance was taken at wavelength of 525 nm. The antioxidant activity was calculated using the given Eq. (1).

$$\text{DPPH Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample inoculate with } \textit{LP.plantarum} \text{ fermented medium})}{\text{Absorbance of control}} X100 \quad (1)$$

### *DMPD antioxidant activity*

The DMPD (N, N-dimethyl, p-phenylendiamine) antioxidant activity was estimated using the protocol given by Fogliano et al. [25]. Different volumes (100 µl, 200 µl, 300 µl) of fermented BLF juicewere added to 2 ml of DMPD solution, and final volume was adjusted to 3 ml by adding distilled water. The reaction mixture was incubated at 37 °C for 10 min and the absorbance was measured at 505 nm. The DMPD antioxidant activity was estimated using the given Eq. (2).

$$\text{DMPD Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample inoculate with } \textit{LP.plantarum} \text{ fermented medium})}{\text{Absorbance of control}} X100 \quad (2)$$

### Antibacterial activity

Anti-bacterial activity of *Lp. plantarum* fermented BLF juice was screened by agar well-diffusion assay against three pathogenic bacterial strains, such as *Escherichia coli* (MTCC-77) (Gram -ve), *Klebsiella pneumoniae* (MTCC-109) (Gram -ve), and bacteria *Staphylococcus aureus* (MTCC-737) (Gram + ve). Nutrient Agar plates were swabbed with 100 µL of a 24 h fresh culture of the above-mentioned bacterial strains. The wells were made using a sterile cork borer, and the lower portion of the wells was sealed using molten agar medium. Different concentrations (100, 200 µL) of 120 h fermented juice extract samples and crude fruit juice were used to assess antibacterial activity. The plates were kept for 10 min to allow the extract to diffuse into the agar. After incubating the plates for 24 h at 37 ℃, the plates were observed to measure the inhibition zone. The zones of inhibition were measured using the scale, and the data were represented in millimeters. The studies were conducted in triplicate, and the average value was calculated for the ultimate antibacterial activity.

### Fourier transform infrared spectroscopy analyses

The functional groups, chemical structures of the compounds present in the BLF fermented lacto-juice, and crude BLF juice (*i.e.*, non-fermented *B. lanzan*) were recorded using Alpha attenuated total reflectance-FTIR spectrophotometer (Alpha ATR-FTIR, Bruker, Germany). The vibrations occurring in the bonds were measured by taking the samples at 128 scans and 2 $cm^{-1}$ resolution up to the range of 4000–500 $cm^{-1}$ [19].

### Scanning electron microscope analysis

*Lp. plantarum* fermented BLF juice was lyophilized and then sprayed with gold nanoparticles. The morphology and attachment of the bacterial cells in the fermented sample was observed using SEM (ZEISS Gemini SEM300 SEM). A voltage of 10 kv was used to determine the surface structure of the *B. lanzan* fermented sample [26].

### GC-MS analysis

The volatile compounds present in BLF lacto-juice were estimated using GC-MS-QP2010 ultra with ECD, FID, AOC-20i, and HT3 Headspace analyser. The VFNAX MAS column (L = 30 m and D = 0.25 mm) was fitted with a GC-MS system and injected with a carrier gas (He, 99.99% purity) with a flow rate of 1.5 ml/min. The temperature of the kiln was maintained at 90 °C for 2 min and further increased to 220 °C. The volatile organic compounds that are present in the fermented *B. lanzan* lacto-juice were ionized at 70 eV ionization energy and a temperature of 230 °C. The mass-to-charge electron (m/z) range 0–500 was analyzed, and all the data were collected using chromatograms at the change of retention time. The mass spectra corresponding to different VOCs were plotted and related to the spectral information present in the National Institute of Standards and Technology repository [19].

## Results and discussion

### Screening of phytochemicals of *B. lanzan* fruit juice

The occurrence of phytochemicals in crude BLF juice was studied to determine its biological and pharmaceutical facets. The results showed that tannin, saponin, steroid, glycoside, reducing sugar, phytosterols, triterpenoids, terpenoids, phlobatannis, leuconthocyanins, and phenolic compounds were present in the BLF fruit juice. The presence of tannin and other phytochemicals suggested that it may have antioxidant, antimicrobial, and anti-inflammatory properties [27]. The other phytochemicals, such as flavonoids, cardiac glycosides, and quinones, were found to be absent in the fruit juice (Table 1; Fig. 2), indicating a selective phytochemical profile of the BLF juice. The presence of phenolic compounds found in the presence study reveal potential

of the fruit extract towards various biological properties such as antiapoptotic, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection, and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [28, 29]. The preliminary phytochemicals analyses for BLF juice can possess pharmaceutical properties which could be useful for health drinks [30].

### Fermentation of *B. lanzan* using starter culture

In the current study, three different probiotic strains, such as *Lp. plantarum, Lvb. brevis, and L. rhamnosus* were used to ferment the fruit juice of *B. lanzan* in order to screen the efficient strain for lacto-juice production. The fermented fruit juice is usually prepared to increase the levels of vitamins, minerals, and nutritive value that are readily available for absorption in our body [31]. The changes in crude protein, pH, total sugar and LA of the fermented BLF juice were estimated after five different incubation periods (24 h, 48 h, 72 h, 96 h, 120 h) and the results are given below.

**Table 1** Phytochemical screening of *B. lanzan* fruit juice

| Name of the test | Results |
|---|---|
| Tannin | + |
| Saponin | + |
| Steroid | + |
| Glycoside | + |
| Flavonoid | - |
| Alkaloid | - |
| Reducing sugar | + |
| Cardiac glycosides | - |
| Phytosterols | + |
| Quinones | - |
| Triterpenoids | + |
| Terpenoids | + |
| Phlobatannis | + |
| Leuconthocyanins | + |
| Phenolic compound | + |

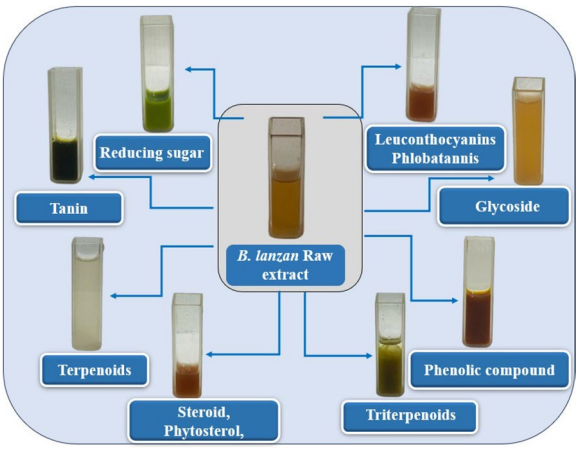



**Fig. 2** Phytochemical analysis of *B. lanzan* fruit extract

### Estimation of crude protein of both the fermented and non-fermented BLF juice

The change in proximate composition, i.e., crude protein of BLF juice after different hours of fermentation, was estimated and presented in Table 2 along with their statistical values and Fig. 3. ANOVA showed a significant effect of *Lactobacillus* strains on the production of protein at all incubation periods except 0 h ($p < 0.001$). As the fermentation period increased from 24 to 120 h ($F(5,36) = 1991.8$ $p < 0.001$), the crude protein content increased. *Lp. plantarum* fermented BLF lacto-juice showed an increase in concentration of crude protein from $5.71 \pm 0.23$ to $38.45 \pm 0.76$ mg/ml ($P < 0.05$). Similarly, *Lvb. brevis* and *L. rhamnosus* fermented lacto-juice showed an increase in concentration of protein from $5.71 \pm 0.23$ to $31.00 \pm 0.05$ and $23.58 \pm 0.16$ ($P < 0.05$), respectively. *Lp. plantarum* exhibited the highest value of protein at 72-120 h, while *L. rhamnosus* consistently showed the lowest values of protein. The statistically significant differences among strains were calculated using Tukey's post-hoc test, which was represented by different superscript letters (Table 2). This increase in crude protein after fermentation can be attributed to the proliferation of LAB in the accumulation of biomass, or the production of extracellular proteolytic enzymes that release soluble peptides and amino acids, thereby

**Table 2** Protein estimation of *B. lanzan* fruit juice extract during fermentation at different incubation times using *Lp. plantarum, Lvb. Brevis, and L. rhamnosus*

| Incubation period (h) | Protein Estimation (mg/ml) | | |
|---|---|---|---|
| | ***Lp. plantarum*** | ***Lvb.brevis*** | ***L. rhamnosus*** |
| 0 | $5.71 \pm 0.23^{a}$ | $5.71 \pm 0.23^{a}$ | $5.71 \pm 0.23^{a}$ |
| 24 | $14.75 \pm 0.44^{c}$ | $20.70 \pm 0.04^{a}$ | $16.13 \pm 0.24^{b}$ |
| 48 | $25.49 \pm 0.97^{b}$ | $27.72 \pm 0.05^{a}$ | $19.83 \pm 0.53^{c}$ |
| 72 | $29.89 \pm 0.55^{a}$ | $28.14 \pm 0.38^{b}$ | $21.64 \pm 0.33^{c}$ |
| 96 | $33.19 \pm 0.72^{a}$ | $29.22 \pm 0.27^{b}$ | $22.52 \pm 0.29^{c}$ |
| 120 | $38.45 \pm 0.76^{a}$ | $31.00 \pm 0.05^{b}$ | $23.58 \pm 0.16^{c}$ |

± Standard deviation

Significant ($P < 0.001$)

Values in the same row with different symbols showed a significant difference ($P < 0.05$)

a, b, c, mean significant differences

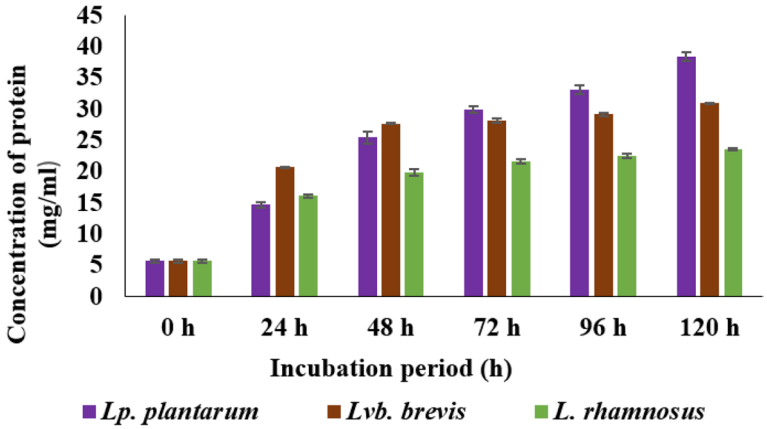



**Fig. 3** Estimation of crude protein present in fermented *B. lanzan* fruit extract

increasing extractable protein levels [32, 33]. The findings of the present study are consistent with a previous report on LA fermentation of malted ragi using *Lp. plantarum*, where protein content increased from 8.81 to 8.64% after LAB fermentation [34]. Thus, fermentation of BLF juice, particularly using *Lp. plantarum*, demonstrated promising potential for developing protein-enriched functional beverages with improved nutritional and functional attributes.

### Estimation of pH of fermented B. lanzan juice

The pH of fermented BLF juice slightly decreased with the increase in the incubation period 24 to 120 h (Table 2). The pH of crude BLF juice (control) was 5.18 ± 0.71. In case of fermentation with *Lp. plantarum* fermented BLF juice, the pH found to decreases with values of 4.88 ± 0.92, 4.85 ± 0.43, 4.83 ± 0.71, 4.82 ± 0.81, and 4.82 ± 0.55, after 24 h, 48 h, 72 h, 96 h, and 120 h of fermentation, respectively. Similarly, *Lvb. brevis* fermented BLF juice also showed slightly decrease in pH from 5.18 ± 0.71 to 4.86 ± 0.45, after a 120 h incubation period. Fermentation of BLF juice with *L. rhamnosus* also showed a similar trend to that of *Lvb. brevis* (Table 3). From the above results, it was found that *Lp. plantarum* showed the highest decrease in pH compared to the other two strains, *Lvb. brevis* and *L. rhamnosus*. This implies better fermentation of the *Lp. Plantarum*. pH is the main factor in preserving and developing the aroma and flavour of many fermented fruit extracts. This acidification not only enhances microbial safety and shelf stability but also plays a crucial role in modulating sensory attributes by improving the quality of the fermented juice.[35]. Most of the LAB grow in a near-neutral pH [36]. The decrease in pH at the beginning of fermentation has great importance for developing texture and quality of fermented products [37]. Decrease in pH during LA fermentation was probably due to accumulation of organic acids, mainly LA [38]. ANOVA analysis of pH value did not show significant changes due to the buffering capacity of the *B. lanzan* juice, even though a significant increase of LA was observed. The previous report on LAB fermentation of pomegranate juice using *Lp. plantarum* PU1, showed no significant decrease in pH due to the buffering capacity of fruit juice, supported the results of the present study [39].

**Table 3** Total pH estimation of *B. lanzan* fruit juice extract during fermentation at different incubation times using *Lp. plantarum*, *Lvb. brevis*, and *L. rhamnosus*

| Incubation period (h) | pH | | |
|---|---|---|---|
| | ***Lp. plantarum*** | ***Lvb. brevis*** | ***L.rhamnosus*** |
| 0 | 5.18 ± 0.71 | 5.18 ± 0.71 | 5.18 ± 0.71 |
| 24 | 4.88 ± 0.92 | 4.87 ± 0.71 | 4.95 ± 0.65 |
| 48 | 4.85 ± 0.43 | 4.86 ± 0.26 | 4.97 ± 0.12 |
| 72 | 4.83 ± 0.71 | 4.86 ± 0.95 | 4.97 ± 0.26 |
| 98 | 4.82 ± 0.81 | 4.86 ± 0.76 | 4.94 ± 0.32 |
| 120 | 4.82 ± 0.55 | 4.86 ± 0.45 | 4.93 ± 0.45 |

± Standard deviation

Values in the same column with different indices showed not significant difference (P > 0.05)

### Estimation of total sugar

The total sugar content (glucose, fructose, and sucrose) of the crude BLF and *Lactobacillus* sp. fermented BLF juice was estimated to determine the change in concentration of sugar before and after fermentation of BLF juice. The total sugar of *Lp. plantarum* fermented BLF juice was estimated to be 147.11 ± 0.37 g/L after 0 h incubation period, and it gradually decreased with the increase

in incubation period. After 120 h of fermentation, the *Lp. plantarum* fermented BLF juice showed a total sugar content of 56.88 ± 0.55 g/L. Similarly, *Lvb. brevis* fermented BLF juice gradually decreased from 143.78 ± 0.71 to 70.34 ± 0.95 with the increase in incubation period from 0 to 120 h of fermentation. The *L. rhamnosus* fermented *B. lanzan* juice showed total sugar content of 65.88 ± 0.23 g/L after a 120 h fermentation period and gradually decreased with the increase in incubation period after 120 h of fermentation (Table 4; Fig. 4). The *Lp. Plantarum* fermented BLF lacto-juice showed the highest decrease in sugar concentrations of 56.88 ± 0.55 g/L as compared to *Lvb. brevis* and *L. rhamnosus* fermented BLF lacto-juice with sugar concentrations of 70.34 ± 0.95 and 65.88 ± 0.23 g/L, respectively. *Lp. Plantarum* showed amylolytic activity, which helps in the production of various organic acids through its fermentation. The ANOVA analyses of obtained sugar data revealed that fermentation time ($F_{(5,36)} = 79.41$; $P < 0.001$) and different bacterial strains ($F_{(2,36)} = 92.31$; $P < 0.001$) had a highly significant effect on total sugar concentration. The *Lp. plantarum* fermented vegetables (cucumber, carrot, and radish) showed decrease in sugar concentration with the increase in fermentation time supported the findings of the present study[40]. In the present study, the fermented *B. lanzan* juice did not transform all the total sugar into LA. This was probably due to the consumption of sugars by *Lp. plantarum* for their growth and development [41].

**Table 4** Total sugar concentration of *B. lanzan* juice extract during fermentation at different incubation times using *Lp. plantarum, Lvb. brevis, and L. rhamnosus*

| Incubation period (h) | Total sugar concentration g/L of fruit juice | | |
|---|---|---|---|
| | *Lp. plantarum* | *Lvb. brevis* | *L. rhamnosus* |
| 0 | $147.11 \pm 0.37^{a}$ | $143.78 \pm 0.71^{a}$ | $140.78 \pm 0.71^{a}$ |
| 24 | $136 \pm 0.32^{b}$ | $137 \pm 0.65^{b}$ | $139.85 \pm 0.65^{a}$ |
| 48 | $112.14 \pm 0.07^{c}$ | $135.75 \pm 0.26^{b}$ | $158.68 \pm 0.26^{a}$ |
| 72 | $81.28 \pm 0.32^{c}$ | $150.77 \pm 0.51^{a}$ | $140.39 \pm 0.51^{b}$ |
| 96 | $75.91 \pm 0.40^{c}$ | $112.10 \pm 0.14^{a}$ | $102.73 \pm 0.14^{b}$ |
| 120 | $56.88 \pm 0.55^{c}$ | $70.34 \pm 0.95^{a}$ | $65.36 \pm 0.95^{b}$ |

Values: mean ± SD

Significant: $P < 0.001$

Different superscript letters within the same row indicate significant differences among bacterial strains (ANOVA followed by Tukey's post-hoc test, $p < 0.05$)

a, b, c: significant differences

### Estimation of lactic acid in fermented juice

LA is the core organic acid formed by *Lactobacillus* strains during their fermentation of various foods [15]. Initially, the LA concentration of BLF juice was found to be 3.14 ± 0.24 g/L. After fermentation for 120 h with *Lp. plantarum*, *Lvb. brevis*, *L. rhamnosus*, showed the concentration of LA increased in the range of 16.78 ± 0.30 g/L, 12.78 ± 0.45 g/L, 14.98 ± 0.11 g/L, respectively (Table 5; Fig. 5). The strain *Lp. plantarum* fermented BLF juice showed the highest concentration of LA as compared to the other two strains, *Lvb. brevis*, *L. rhamnosus.* The concentration of LA was found to increase due to the conversion of fermentable sugars into LA by the rapid activity of *Lp. Plantarum* as evident from the decrease in sugar. The ANOVA analyses of the obtained LA data showed that incubation time had a highly significant effect on LA production ($F_{(5,36)} = 412.87$, $p < 0.001$), and bacterial strain also significantly influenced LA concentration ($F_{(2,36)} = 18.64$, $p < 0.001$). Karovicova and Kohajdová [42] also reported the highest production of LA in green

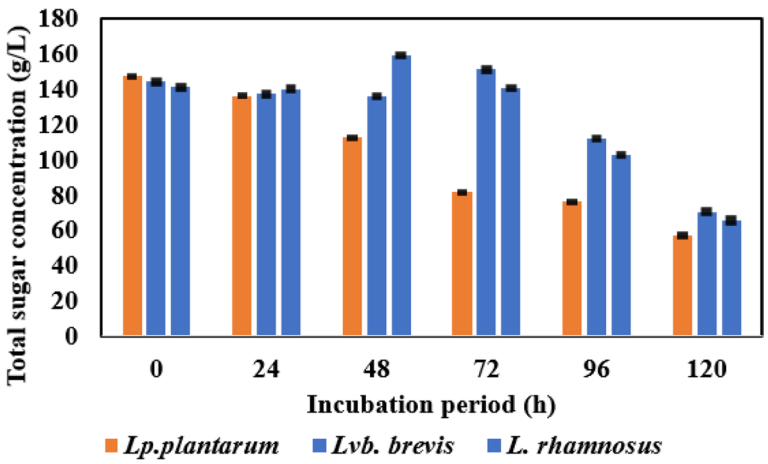



**Fig. 4** Estimation of total sugar of *Lactobacillus* strains fermented *B. lanzan* Lacto-juice

**Table 5** Lactic acid estimation of *B. lanzan* fruit juice extract during fermentation at different incubation times using *Lp. plantarum*, *Lvb. brevis*, and *L. rhamnosus*

| Incubation period (h) | Lactic acid concentration(g/L) | | |
|---|---|---|---|
| | *Lp. plantarum* | *Lvb. brevis* | *L. rhamnosus* |
| 0 | 3.14 ± 0.24[a] | 3.14 ± 0.12[a] | 3.14 ± 0.04[a] |
| 24 | 4.48 ± 0.18[c] | 4.9 ± 0.2[b] | 5.37 ± 0.25[a] |
| 48 | 7.67 ± 0.4[b] | 7.86 ± 0.25[a] | 7.08 ± 0.03[c] |
| 72 | 8.44 ± 0.09[b] | 9.55 ± 0.19[a] | 8.08 ± 0.33[c] |
| 96 | 13.86 ± 0.12[a] | 13.43 ± 0.23[c] | 13.76 ± 0.22[b] |
| 120 | 16.78 ± 0.3[a] | 12.78 ± 0.45[c] | 14.98 ± 0.11[b] |

± Standard deviation

Significant: $P < 0.001$

Different letters indicate significant differences among bacterial strains (ANOVA followed by Tukey's post-hoc test, $p < 0.05$)

Different symbol a,b,c indicate significant differences

pea, carrot, celery, and onion salads using *Lactobacillus* sp. as an inoculant. Gardner et al. [43] have conducted their study using various LAB for the fermentation of cabbage, carrot, and beet-based products. It was evident from above report that a starter culture consisting of *Lp. plantarum* NQ 312, *Pediococcus acidilactici* AFERM 772, and *Leuconostoc mesenteroides* BLAC fastened the fermentation and produced higher LA that supports the findings of the present study.

### Antimicrobial activity of fermented BLF juice against human pathogenic bacteria

The fermented BLF juice showed antibacterial activity against three tested pathogenic bacterial strains, *S. aureus* (MTCC-737), *K. pneumoniae* (MTCC-109), and *E. coli* (MTCC-77). These antimicrobial activities were measured in terms of the inhibition zone produced against their pathogenic strains. Various amounts of (100, 200 µl) fermented BLF juice were used to analyze the antimicrobial responses. An antibiotic (Kanamycin) was taken as the positive control in this assay. This antibiotic showed 19.12 ± 0.55 mm, 5.6 ± 0.33 mm, and 5.1 ± 0.33 mm inhibition zones for *S. aureus*, *E. coli*, and *K. pneumoniae,* respectively. The non-fermented BLF juice showed an inhibition zones of 13 ± 0.57 mm for *S. aureus*, 2.60 ± 0.88 mm for *E. coli,* and 2.66 ± 0.33 mm for *K. pneumoniae.* Similarly, the 100 µl of the fermented BLF juice showed an inhibition zones of 18.66 ± 0.066 mm for *S. aureus,* 1 ± 0.57 mm for *E. coli,* and 1.66 ± 0.33 mm for *K. pneumoniae.* Moreover, the 200 µl of the fermented sample showed an increased inhibition zones of 21 ± 0.57 mm for *S. aureus,* 1.2 ± 0.57 mm for *E. coli,* 2 ± 0.33 mm for *K. pneumoniae* (Fig. 6A). It was observed that 200 µl of the fermented sample showed a higher inhibition zone compared with the antibiotic (kanamycin). Inhibition zone data on the antimicrobial activity of *B. lanzan* fruit extract was found to be between 7–11 mm for gram-positive bacteria and between 3-9 mm for gram-negative bacteria [44, 45]. The antimicrobial activity of *Lp. plantarum* fermented BLF juice is reported for first time in the present study as no such studies conducted earlier.

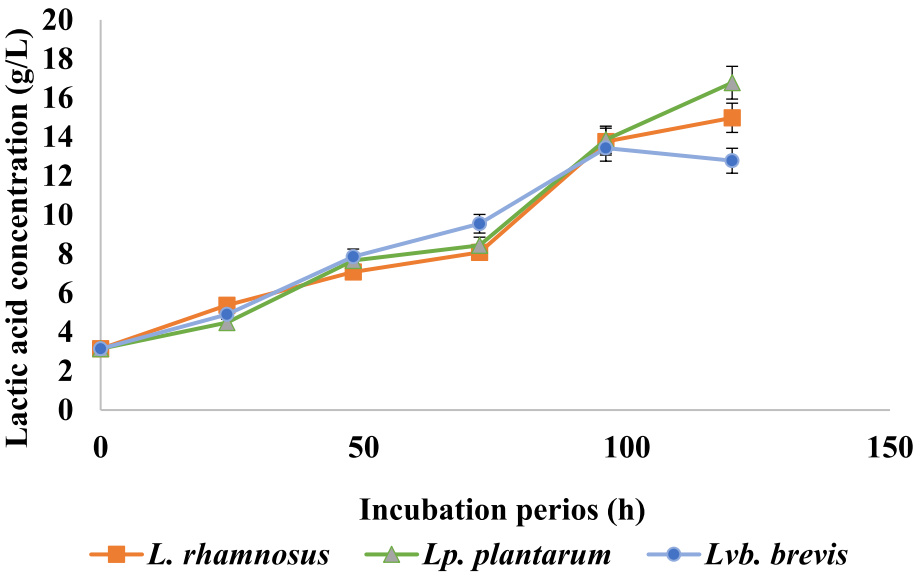



**Fig. 5** Estimation of LA using three *Lactobacillus* strains fermented *B. lanzan* fruit juice

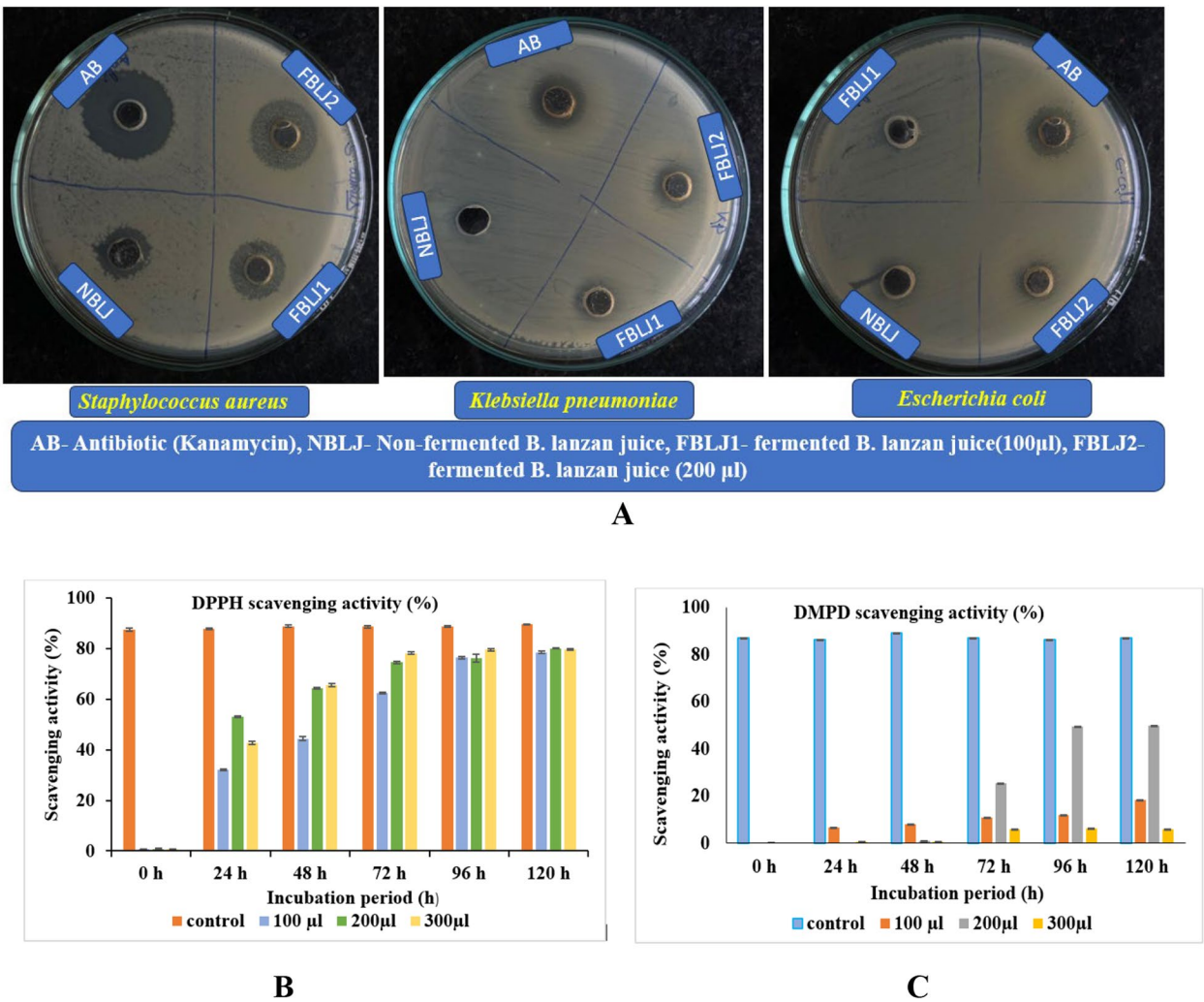



**Fig. 6** Antimicrobial and antioxidant activity of *Lp. plantarum* fermented *B. lanzan* fruit juice (**A**) anti-microbial activity (**B**) DPPH (**C**) DMPD scavenging activity

## Antioxidant activity

### *DPPH antioxidant activity*

The antioxidant activity of BLF juice before and after fermentation was estimated to determine the free radical scavenging activity. It was generally observed that the fermented BLF juice showed higher scavenging activity as compared to the non-fermented BLF juice. Various amounts (100, 200, 300 µl) of fermented samples were assayed to determine their scavenging activity. The 100 µl of fermented *B. lanzan* at 120 h showed 78.57 ± 0.45% of scavenging activity. The 300 µl of 120 h fermented samples showed the highest scavenging activity of 80.08 ± 0.13% as compared to the other two doses, 100 µl and 200 µl fermented samples with scavenging activities of 78.57 ± 0.45% and 79.65 ± 0.39%, respectively. The fermented BLF juice was comparable with the standard chemical, ascorbic acid, with scavenging activity of 89.54 ± 0.20% (Fig. 6B).

### *DMPD antioxidant activity*

The DMPD antioxidant activity of BLF juice pre and post fermentation was also estimated to determine the free radical scavenging activity. Similar to DPPH scavenging activity, in DMPD antioxidant assay, it was also observed that the fermented BLF juice showed higher scavenging activity as compared to the non-fermented sample. Various amounts (100, 200, 300 µl) of fermented BLF juice were assayed to determine the scavenging activity. The 100 µl dose of the fermented BLF juice at 120 h of fermentation showed 18.22 ± 0.012% of scavenging activity. The 200 µl dose of 120 h fermented BLF juice showed the highest scavenging activity of 49.71 ± 0.020% as compared to the other two doses, 100 µl and 300 µl fermented samples,

with scavenging activity 18.22 ± 0.012% and 6.05 ± 0.017%, respectively. The scavenging activity of fermented BLF juice was comparable with the standard chemical ascorbic acid, which showed scavenging activity of 86.99 ± 0.017% (Fig. 6C). The increased antioxidant activity may be due to the change in composition of bioactive compounds after fermentation. According to Lin and Chang [46] (2000), the presence of enzymatic antioxidants like NADH and NADPH, as well as peptides in of LAB cells, can contribute to increased antioxidant activity.

### Scan electron microscopy analysis

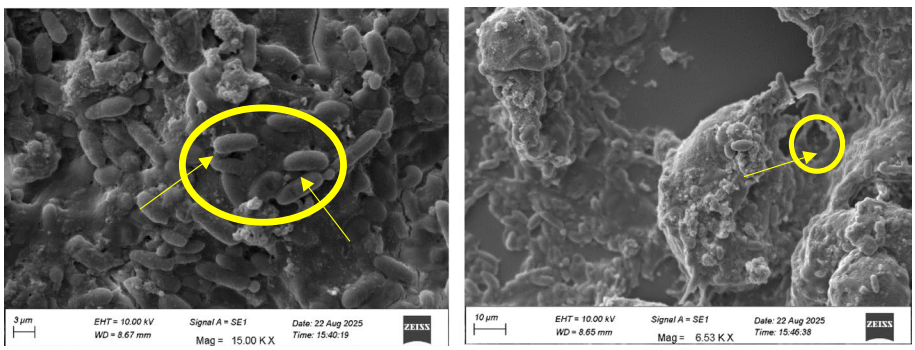
The adhesion and morphological changes of *Lp. plantarum* on BLF lacto-juice were observed by using SEM analysis. The 10 kV electron beam in the SEM was used to observe the attachment of *Lp. plantarum* to the fermented BLF juice. The results showed the attachment of *Lp. plantarum* to the surface of fermented *B. lanzan* lacto-juice sample (Fig. 7) that supported the results obtained by previous other researchers who have examined the interactions of *Lactobacillus* with fermented roots, juice, and tubers [19, 47]. This is evidence from the fermented BLF juice that a generous microbe present as a transporter of, inherent nutritional properties.

### Fourier transform infrared analysis of *B. lanzan* Lacto-juice

Fermentation-induced changes in juice samples were studied by comparing their functional groups before and after fermentation using FTIR spectroscopy. In a recent study, ATR-FTIR spectroscopy was employed to analyse juice sample after 120 h of fermentation. The resulting spectra, which were interpreted based on existing literature, revealed several key absorbance peaks. A prominent broad peak, observed between 3290.93 to 1630.52 $cm^{-1}$, was linked to OH stretching (Fig. 8). Zeng et al. [48] have found that this particular OH- peak signifies the presence of various compounds containing hydroxyl groups, such as alcohols, phenols, or carboxylic acids (COOH).

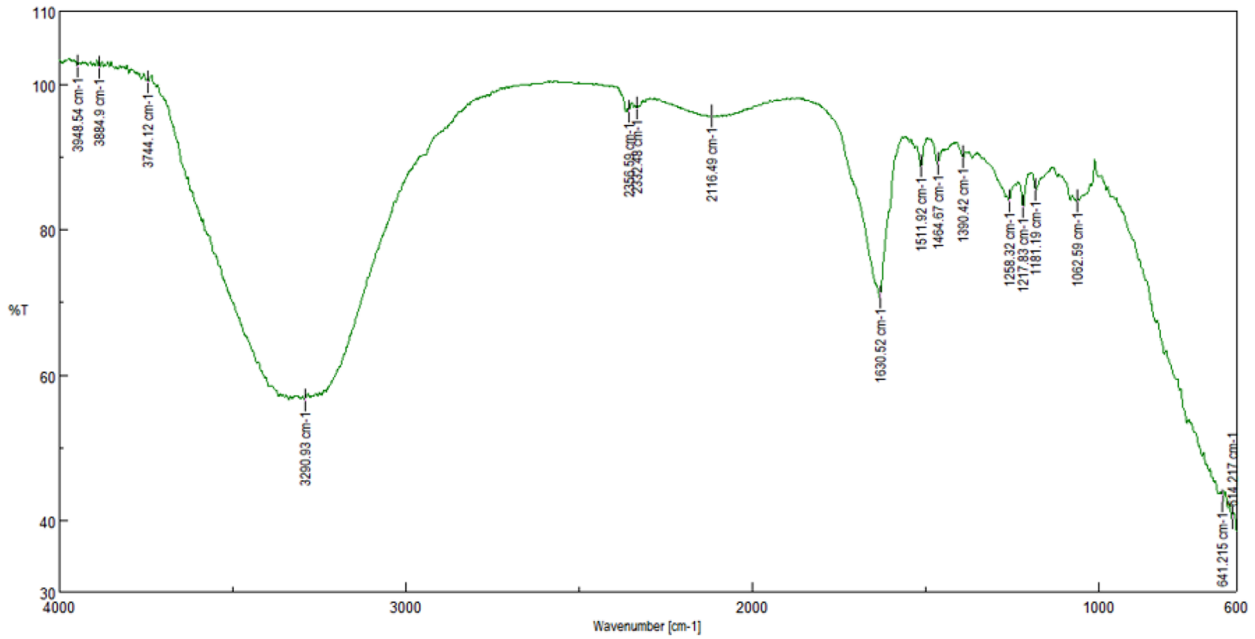
### Gas chromatography-mass spectrometry analysis of B. lanzan Fruit juice

The identification of numerous compounds present in the fermented BLF juice has been made possible by the GC-MS analysis by comparing their mass spectra. Fatty acid esters, fatty acids, alcohols, aldehydes, and steroids were the chemical classes that were identified from GC-MS analyses. Specific parameters such as peak area, retention time (RT), height, and m/z (mass-to-charge) ratio were used in determining each molecule (Fig. 9). The compound (Cyclopentane) has a maximum peak area of 62.71% (m/z = 2.73). Other compounds such as 5-nonynoic acid, 2-bromo-4,6-difluoroaniline, butyric acid, 3-thiophenamine, heptanoic acid, l-gala-l-ido-octose, naphthalene, ethanol, 2-nonenoic acid, 1,3-dimethoxypropan-2-yl ol, 2H-1,2,3,4-tetrazole-2-acetonit, galactonic phenyl, benzene, androst-9(11)-en-17-on, 3-chloro-N-[2-methyl-4(3H)-ox, 5-nonenoic acid, 4-amino-3-phenyl-2-thioxo-2,3-di, cysteine, trans-3-cyclopropyl-7-(2)-metho, cycloheptasiloxane, and LA were also found at different retention time and peak areas (Table 6). It has been reported that GC-MS analysis of different parts of the BLF juice showed the presence of important pharmacological compounds used in various pharmaceutical and nutraceutical industries [49]. The fermented BLF juice, produced by probiotic bacteria, was useful for enhancing the flavour, bioactivity, and chemical profile of the juice [50]. This is the first report on the GC-MS analysis of *Lp. Plantarum* fermented BLF juice, as no data were reported on fermented juice of BLF to date.




**A** **B**

**Fig. 7** SEM photograph (**A**, **B**) shows the topography and attachment of rod-shaped *Lp. plantarum* (MTCC-1407) to the surface of *B. lanzan* lacto-juice. Circle with arrows = *Lp. plantarum* (MTCC-1407) attachment on the sample



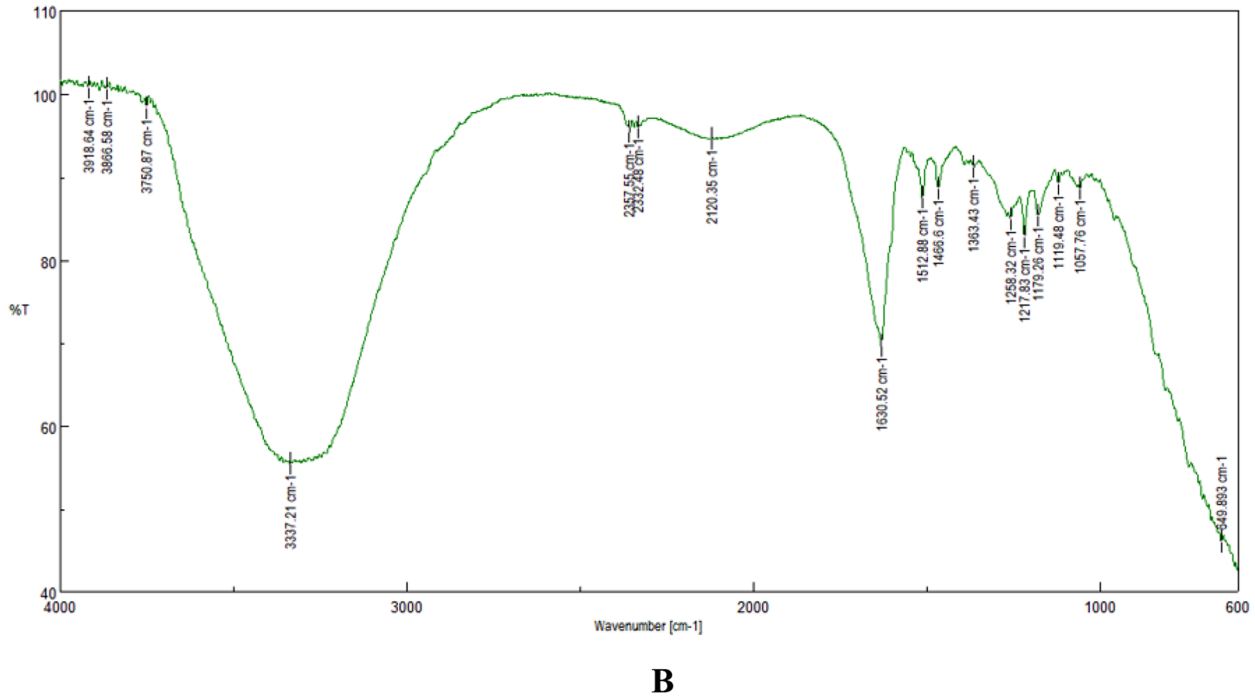




**Fig. 8** FTIR analysis of (**A**) (raw extract), (**B**) (*Lp. plantarum* fermented Lacto-juice) of *B. lanzan*

## Conclusion

The *Lp. plantarum* is a promising strain extensively used for the production of fermented fruit juice with high therapeutic potential. The LAB fermentation study on BLF juice using *Lp. plantarum* as the choicest strain showed the presence of a good amount of crude protein with better source of nutritional, antimicrobial, and antioxidant activities. The phytochemical screening of

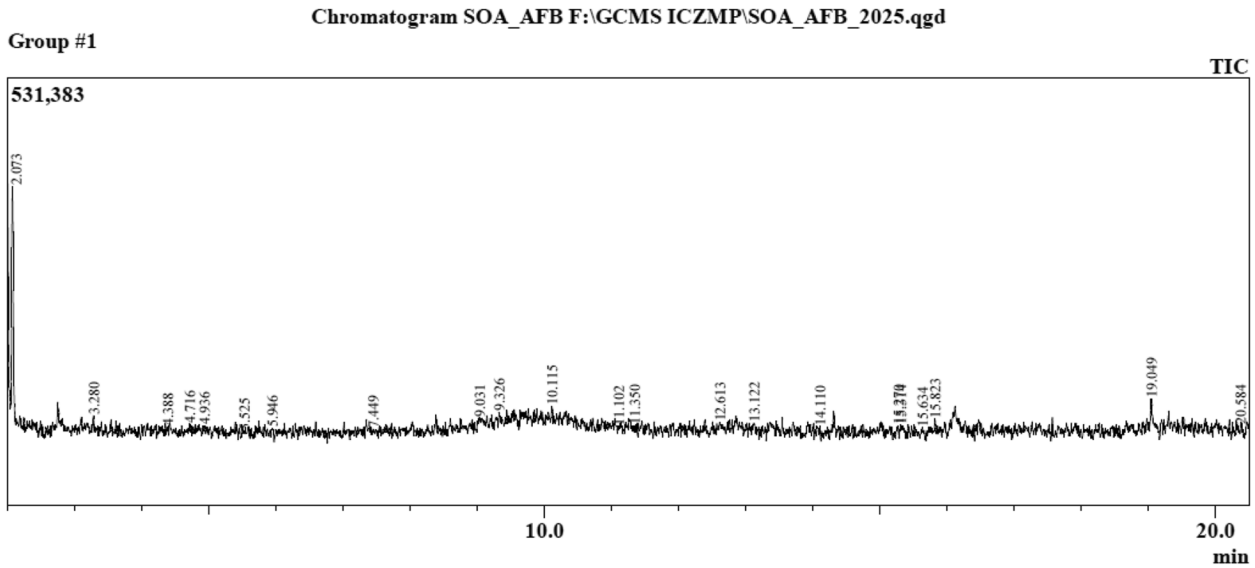



**Fig. 9** GC-MS analysis of *Lp. plantarum* fermented *B. lanzan* fruit juice

**Table 6** Various compounds estimated in GC–MS analysis of fermented *B. lanzan* lacto-juice

| No of Peaks | Retention Time (min) | Peak area (%) | Height (%) | Compounds |
|---|---|---|---|---|
| 1 | 2.073 | 62.71 | 55.99 | Cyclopentane |
| 2 | 3.280 | 2.51 | 3.55 | 5-Nonynoic acid |
| 3 | 4.388 | 0.34 | 1.32 | 2-Bromo-4,6-difluoroaniline |
| 4 | 4.716 | 1.19 | 1.85 | Butyric acid |
| 5 | 4.936 | 1.45 | 1.51 | 3-Thiophenamine |
| 6 | 5.525 | 0.33 | 0.52 | Heptanoic acid |
| 7 | 5.946 | 3.89 | 2.72 | l-Gala-l-ido-octose |
| 8 | 7.449 | 0.62 | 0.89 | Naphthalene |
| 9 | 9.031 | 0.89 | 1.11 | Ethanol |
| 10 | 9.326 | 3.09 | 2.92 | 2-Nonenoic acid |
| 11 | 10.115 | 2.58 | 3.17 | 1,3-Dimethoxypropan-2-yl ol |
| 12 | 11.102 | 0.57 | 1.16 | 2H-1,2,3,4-Tetrazole-2-acetonit |
| 13 | 11.350 | 0.55 | 1.27 | beta-Galactonic phenyl |
| 14 | 12.613 | 0.41 | 0.89 | Benzene |
| 15 | 13.122 | 2.10 | 1.89 | Androst-9(11)-en-17-on |
| 16 | 14.110 | 1.01 | 2.69 | 3-Chloro-N-[2-methyl-4(3H)-ox |
| 17 | 15.270 | 1.74 | 2.10 | 5-Nonenoic acid |
| 18 | 15.314 | 0.63 | 1.34 | 4-Amino-3-phenyl-2-thioxo-2,3-di |
| 19 | 15.634 | 0.66 | 1.02 | Cysteine |
| 20 | 15.823 | 2.32 | 3.25 | *trans*-3-Cyclopropyl-7-(2-metho |
| 21 | 19.049 | 0.71 | 1.68 | Cycloheptasiloxane |
| 22 | 20.585 | 9.69 | 7.17 | Lactic acid |

*B. lanzan* fruit juice showed the presence of numerous phytochemicals, which could contribute to health benefits in nutritious health drinks. The *Lactobacillus* fermented BLF fruit juice could be a potential valuable beverage for the improvement of gut health. The GC-MS analysis of fermented BLF juice revealed the presence of various pharmaceutically important compounds, which are responsible for improving the health and nutritional status of the fermented beverage. Furthermore, in vivo cell line evaluation of this fermented juice could also reveal

the cytotoxicity effect, while clinical studies are required to substantiate the health benefits of fermented *B. lanzan* beverages. Addressing these aspects of the investigation will strengthen the scientific understanding and support the sustainable utilization of this underexploited fruit resource for highly beneficial health drink and tonic.

**Acknowledgements**
The authors express gratitude to Vice-Chancellor, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar, Odisha, India, for providing the necessary infrastructure facilities to carry out the present research work. The authors extend their gratitude to the Dean of School of Pharmaceutical Sciences (SPS) Department, SOADU, for FTIR analyses and interpretation of the data. The author(s) are also grateful to ITER, SOADU, for the SEM analysis used in the present study. The author(s) extend their gratitude to the Coastal Management cell, Central lab and Regional Office, Odisha State Pollution Control Board, Bhubaneswar, Odisha, India, for offering technical support during the GC-MS analysis conducted in this study.

**Authors' contributions**
LR conducted a significant portion of the experiments, examined the outcomes, analysed the data, wrote the results with pertinent prior research, and made substantial contributions to both the composition of the initial manuscript and its formatting**.** AS and SR helped examine the outcomes, analyse the data, cross-validate the results of the present work, and helped in manuscript writing. RCR and HNT provided comprehensive oversight for the entire project, formulated the research concept, and provided guidance for manuscript composition and editing. The manuscript has been reviewed and approved by all authors.

**Funding**
Open access funding provided by Siksha 'O' Anusandhan (Deemed To Be University) This work was financially supported by the SOA Ph.D. research fellowship, Shiksha 'O' Anusandhan, deemed to be University to the author LR, for conducting her research work.

**Data availability**
All data generated or analyzed during this study are included in this article.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Competing interests**
The authors assert that there are no conflicts of interest to declare.

Received: 10 November 2025 Revised: 24 January 2026 Accepted: 10 March 2026
Published online: 29 April 2026

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