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Biofunctional Characterization of Fruit Yoghurt with *Prunus napaulensis* from North Eastern Region of India

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ABSTRACT

Background: *Prunus napaulensis*, a wildly growing fruit of northeast India remains underutilized although widely consumed for health benefits. The present study attempted at development of bio-functional yoghurt with *Prunus napaulensis* fruit pulp.

Methods: Fruit yoghurt was formulated with optimized level of *Prunus napaulensis* pulp (% w/v) and culture of *Lactobacillus delbrueckii subsp. bulgaricus* (NCDC004) and *Streptococcus thermophilus* (NCDC075) (% w/v). The fruit yoghurt was determined for its phytochemical content and analyzed for its antioxidant activity (DPPH and FRAP assays), antidiabetic activity (α-amylase and α-glucosidase inhibition), anti-inflammatory activity (protein denaturation and protease inhibition) and antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*.

Result: The incorporation of *Prunus napaulensis* in fruit yoghurt, as compared to control yoghurt, exhibited significant increase in the phytochemical content as well as its bioactivity properties. Fruit yoghurt exhibited significantly higher ($p<0.05$) antioxidant activity for DPPH and FRAP assay compared to those of control yoghurt. Antidiabetic activity in fruit yoghurt was demonstrated a significantly higher inhibition ($p<0.05$) of α-amylase and α-glucosidase enzymes. Anti-inflammatory activity was higher as observed from a significantly higher per cent ($p<0.05$) of protein denaturation in fruit yoghurt than control yoghurt. Antimicrobial activity was significantly higher against *Escherichia* coli and *Staphylococcus aureus* in fruit yoghurt than control yoghurt ($p<0.05$). These findings proved that the incorporation of *Prunus napaulensis*, an indigenous fruit of northeast India could be exploited in the development of biofunctional yoghurt with health benefits targeted on chronic diseases of public health threat.

Key words: Antidiabetic activity, Anti-inflammatory, Antimicrobial activity, Antioxidant activity, Fruit yoghurt, *Prunus napaulensis.*

INTRODUCTION

With the growing interest in human health through sustainable diets and culturally rooted food systems, functional foods gain importance with an integration of nutritional science, traditional knowledge and public health innovation (Sawant *et al*., 2025). Functional foods provide both nutritional and health benefits and hence can be an appropriate approach to mitigate public health threating chronic conditions such as diabetes, cardiovascular disease and inflammation-related disorders (Abdi-Moghadam *et al.*, 2023).

Yoghurt, a dairy product is nutritionally dense with lipids, proteins, B vitamins, bioavailable calcium (Abdi-Moghadam *et al*., 2023; Gahruie *et al*., 2015; Ribeiro *et al*., 2021), functionally contains peptides, probiotics (Ghasempour *et al*., 2020) and therapeutically recommended for lactose intolerance (Abdi-Moghadam *et al*., 2023; Ribeiro *et al*., 2021). Aqueous and fat medium in yoghurt solubilize water and fat soluble nutrients for bioavailability and hence yoghurt can be exploited as a suitable delivery system for bioavailability of micro nutrients from fruits through the formulation of fruit yoghurt (Deepa *et al*., 2016; Ranasinghe *et al*., 2021).

Prunus napaulensis (Ser.) Steud also known as Himalayan cherry, Khasi cherry or Sohiong belongs to the Rosaceae family shares similarities with plum, peaches and cherries. It is an indigenous fruit, originated in eastern Himalayan foothills and grown wildly in Jainta and Khasi hills of Meghalaya, Assam, Nagaland, Manipur, Tawang and Dirang regions of Arunachal Pradesh in Northeast India (Rymbai *et al*., 2016). In general, Prunus fruits are locally consumed fresh and minimally processed into value-added products like squash, jelly and wine (Kashyap *et al*., 2022). However, it is classified to be underutilized in India along with the significant post-harvest losses due to inadequate processing techniques, limited storage facilities as well as remain largely unknown outside of Northeast India (Rymbai *et al*., 2016). Micronutrient and bioactive rich

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potential of fruits in reddish-purple hue (Kashyap *et al*., 2022), highly perishable *Prunus* fruits hold promising potential for valorization in to value-added functional food (Aparna *et al*., 2018). Although previous studies have reported the bioactive properties and nutritional enhancement of yoghurt through fortification with cultivated fruits such as pomegranate, persimmon and black mulberry (Bakhti *et al*., 2024; Bchir *et al*., 2020; Durmus *et al*., 2021; Kanca *et al*., 2024) underutilized fruits like *Prunus napaulensis* remain largely unexplored. Recent literatures on fruit enriched yogurts are largely focused on commercially established supply and well characterized fruits (Al-aswad and Shehata, 2025) overlooking the bioactive potential of the wild fruits with dual purpose of valorization of local biodiversity and development of functional food as relevantly emphasized in recent reviews calling for greater attention to research on underutilized fruits and their by-products (Priyashantha *et al*., 2025). The present study was aimed at the formulation of biofunctional yoghurt with antioxidant, antidiabetic, anti-inflammatory and antimicrobial activities with the incorporation of *Prunus napaulensis*.

MATERIALS AND METHODS

Preparation of fruit yoghurt

Fruit yoghurt with *Prunus napaulensis* was prepared with the incorporation of fruit pulp of *Prunus napaulensis* in to standard yoghurt formulation (Plate 1) (Emam and El-Nashar, 2022). Milk was pasteurized to 90°C for 10 min following the inoculation with 1.5% (w/v) of combined culture of *Lactobacillus delbrueckii subsp. bulgaricus* (NCDC004) and *Streptococcus thermophilus* (NCDC075) at 39.5°C for 6 h until setting of yoghurt with pH of 4.6±0.1. The fruit pulp of *Prunus napaulensis* (7% (w/v)) was added in to set yoghurt and stirred thoroughly to obtain stirred fruit yoghurt which was packed in sterile container. The level of fruit pulp of *Prunus napaulensis* and inoculum were optimized through response surface methodology in the previous phase of study (Kashung *et al*., 2025).

Preparation of yoghurt extracts

Yoghurt extracts were prepared according to the methods described by Ghasempour *et al*. (2020) and Shori (2020) with slight modifications. 10 g of yoghurt samples were homogenized with 2.5 mL of distilled water. The mixture was adjusted to the pH of 4±0.1 with 0.1 M hydrochloric acid followed by centrifugation at 5,000 rpm for 10 min at 4°C. The supernatant was neutralized to pH 7±0.1 with 0.5 M NaOH. The supernatant was re-centrifuged at 4000 rpm for 10 min at 4°C. The final supernatant was collected and analyzed for *in vitro* bioactivities.

Determination of phytochemical content

Total flavonoid content of the extracts was determined according to the aluminum chloride colorimetric assay as described by Shraim *et al*. (2021). Folic-Ciocalteu assay method was used to determine the total phenolic content of the extracts as described by Nupur *et al*. (2022) and the result was expressed as mg gallic acid equivalent (mg GAE/g). Total alkaloids of the extracts was determined according to the method described by Koomson *et al*. (2018). The result was expressed as mg of AE/g of the extract.

Determination of antioxidant activity

The DPPH radical scavenging activity was determined according to the method reported by Ali *et al*. (2021). Sample concentrations of 20, 40, 60, 80 and 100 µg/mL were added with 3 mL of 0.004% solution of DPPH. The mixture was incubated for 30 min at 37°C and the absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated from the following equation:

$$\text{Scavenging activity} = \frac{A - B}{A} \times 100$$

Where,
A= Absorbance of the control.
B= Absorbance of the sample.

FRAP assay was performed using the method reported by Arcia *et al*. (2024) with slight modifications. 3 mL of FRAP reagent was added to 0.1 mL of sample. The mixture was incubated in water bath at 37°C for 30 min and the absorbance was measured at 595 nm. The results were expressed as ascorbic acid equivalent (AAE) in µL AAE/g.

Determination of antidiabetic activity: α-amylase enzyme inhibition activity

α-amylase enzyme inhibition activity was determined using the method described by Kadali *et al.* (2017) with some changes. Amylase (0.5 mg/mL) was added to samples (20, 40, 60, 80 and 100 µg/mL) along with 1% starch solution and 100 µl of phosphate buffer (pH 6.9) were added to the mixture. The reaction was carried out for 5 min at 37°C and terminated by adding 2 mL of 3, 5-dinitrosalicylic acid reagent. The mixture was heated at 100°C for 15 min and diluted with 10 ml of distilled water in ice bath. The absorbance was measured at 540 nm using a spectrophotometer. The inhibition (%) was calculated using the equation:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where,
A= Absorbance of the control.
B= Absorbance of the sample containing enzyme.

α-Glucosidase inhibition assay

The inhibition of α-glucosidase enzyme was assessed according to the method described by Kadali *et al*., (2017) with some modifications. To the sample (20, 40, 60, 80 and 100 µg/mL), 100 µL of 0.1M phosphate buffer (pH 6.9), 100 µL of α-glucosidase solution (1 unit/mL) were added.

The mixture was pre-incubated for 5 min at 25°C, followed by the addition of 100 µl of p-nitrophenyl-α-D-glucopyranoside (5 Mm) and incubated for 10 min at 25°C. The absorbance was measured at 405 nm and compared to a control sample containing 100 µL buffer. The inhibition (%) was calculated using the equation:

$$\text{Inhibition (\%)} = \frac{A - C}{A} \times 100$$

Where,
A = Absorbance of the control.
C = Absorbance of the sample containing enzyme.

Determination of Anti-inflammatory activity: Protein denaturation assay

The effect of protein denaturation was determined according to the method described by Gunathilake *et al*. (2018) with slight modifications. 500 µL of 1% bovine serum albumin was added to the sample (20, 40, 60, 80 and 100 µg/mL) and incubated at 37°C for 10 min then heated at 51°C for 20 min. The absorbance was measured at 660 nm on cooling. Acetylsalicylic acid (Aspirin) was used as control. The inhibition denaturation of protein (%) was calculated using the formula:

$$\text{Inhibition denaturation (\%)} = 100 \times \frac{A1 - B2}{A1}$$

Where,
A1 = Absorbance of control sample.
A2 = Absorbance of test sample.

Roteinase inhibitory activity

The proteinase inhibitory activity of the fruit yoghurt was assessed according to the method reported by Gunathilake *et al*. (2018). The reaction solution (2 mL) was prepared by combining 0.06 mL of trypsin, 1 mL of 20 mM Tris-HCL buffer (pH 7.4) and 1 mL of the test sample with varying concentrations of 20, 40, 60, 80 and 100 µg/mL. The solution was incubated for 10 min at 37°C. Subsequently, 1 mL of 0.8% (w/v) casein was added and re-incubated for 20 min. After incubation, 2 mL of 2M perchloric acid ($HClO_4$) was added to terminate the reaction. The resulting suspension was centrifuged at 8000 rpm for 15 min and the absorbance was measured at 280 nm. The percentage of proteinase inhibition was calculated using the formula:

$$\text{Proteinase Inhibition (\%)} = 100 \times \frac{A1 - A2}{A1}$$

Where,
A1 = Absorbance of control sample.
A2 = Absorbance of test sample.

Determination of antimicrobial activity

The antimicrobial activity of the fruit yoghurt was assessed using agar well diffusion method described by Khanal *et al.* (2020). Gram's positive bacteria: *Bacillus cereus* and *Staphylococcus aureus* and Gram's negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli* were used as representative of pathogenic bacteria. Nutrient agar plates were swabbed with overnight bacterial cultures using a sterile cotton swab. 20, 40 and 60 µg/mL of extracts was added to the 10 mm wells made with a cork borer in the nutrient agar plates. It was then incubated for 24 hours at 37°C. The inhibition zones were measured and the inhibitory activity was determined from the average diameter of inhibition zones.

Data analysis

Experiments were performed in triplicates and reported as mean±SD using SPSS version 25.0. Independent t-test,

1 and 2. Standardization of milk using Gerber method for fat content (3.5%) and Lactometer for estimation of Solid-not-fat (SNF 9.5%). 3. Heating milk up to 60°C and homogenization at 2000 psi (phase I) and 500psi (phase - II). 4. Pasteurization at 90°C for 10 min and cooling at 37±2°C. 5. Inoculation of culture (w/v) at conc. 1.5% (*Lactobacillus delbrueckii subsp. bulgaricus* NCDC 004 and *Streptococcus thermophilus* NCDC 075). 6. Incubation at 39.5°C and yoghurt setting until pH reached to 4.6±0.1. 7. Addition of pasteurized pulp conc. of 7% (w/v). 8. Cooling and storage of control (a) yoghurt and fruit yoghurt (b).

Plate 1: Processing steps in yoghurt setting with the pulp of *Prunus napaulensis.*

one way analysis of variance (ANOVA) and Tukey's Post hoc test were performed to analyse the significance of difference in bioactivities of yoghurt with the impact of incorporation of *Prunus napaulensis* at 5 per cent level of significance.

RESULTS AND DISCUSSION

Phytochemical content

Table 1 reports the phytochemical analysis of the control yoghurt and yoghurt with *Prunus napaulensis* revealing substantial differences in the bioactive compounds. Significant difference was observed in the fruit yoghurt ($p<0.05$) (7.35 mg GAE/g) compared to control yoghurt (1.74 mg GAE/g) in the total phenolic content. Additionally, the flavonoid content in the fruit yoghurt (2.68 mg QE/g) exhibited a significant increase ($p<0.05$) as compared to control yoghurt (0.56 mg QE/g). Similarly, the alkaloid content revealed a marked increase from 0.08 mg AE/g in control yoghurt to 0.4 mg AE/g in the fruit yoghurt ($p<0.05$) indicating a positive transfer of bioactive compounds from the fruit to the yoghurt.

Various studies have reported presence of rich amounts of phenolics, flavonoids and alkaloids in *Prunus napaulensis* fruit with major compounds such as rutin, purpurin, tannic acid, gallic acid and ascorbic acid (Kashyap *et al*., 2022; Shi *et al*., 2023; Swer *et al*., 2016). *Prunus* genus are known for their rich source of phytonutrients such as saponins, alkaloids, terpenoids, flavonoids and phenolic compounds that are known for their pharmacological activities. Plums and other *Prunus* species contains bioactive phenolics compounds namely, flavonoids and phenolic acids that have therapeutic effects (Ben Khadher *et al*., 2023; Katanić *et al*., 2022; Popović *et al*., 2021; Wills *et al*., 1983; Yiğit *et al*., 2009). Typically, plain yoghurt lack significant amounts of flavonoids and phenolic compounds limiting its antioxidative potential. Thus the incorporation of *Prunus napaulensis* fruit enhances the bioactive properties transforming conventional yoghurt to a functional food. The significant increase of the phenolic compounds in the fruit yoghurt is consistent with previous studies reporting incorporation of fruits enhances the phytochemical content of the fermented dairy product (Benzineb *et al*., 2025; Emam and El-Nashar, 2022; Gangwar *et al*., 2016; Jany *et al*., 2024). Bchir *et al*., (2020) observed a significant increase in phenolic content with the incorporation of pomegranate while Durmus *et al*., (2021) reported an increase in total phenolic content in mulberry enriched yoghurt. Similarly, the addition of phenolic extract from apple and black currant has been shown to increase the phenolic content (Sun-Waterhouse *et al*., 2012, 2013).

Antioxidant activity

DPPH radical scavenging activity increased corresponding to the increase in the concentration of samples in a dose dependent manner. Fruit yoghurt exhibited significantly higher DPPH radical scavenging activity higher than those of control yoghurt ($p<0.05$) as shown in Fig 1. In comparison to standard, the fruit yoghurt exhibited 20 per cent of activity of ascorbic acid which was higher than that of control yoghurt. Similar to DPPH radical scavenging activity, the FRAP value was observed to be higher for fruit yoghurt than control yoghurt as notable differences revealed in the antioxidant capacities of the tested samples (Table 1). The fruit yoghurt incorporated with *Prunus napaulensis* and control yoghurt exhibited dose-dependent antioxidant activity 350 µg AAE/g and 200 µg AAE/g respectively. The FRAP assay measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}, which correlates with their overall reducing power and potential to neutralize free radicals.

The moderate antioxidant activity of the control yoghurt can be attributed to the presence of milk protein (Guiné and De Lemos, 2020; Szołtysik *et al.,* 2021) and the starter culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) which have been reported to exhibit antioxidant properties (Citta *et al*., 2017; Szołtysik *et al*., 2021). However, its activity was consistently lower than that of *Prunus napaulensis* fruit yoghurt which can be attributed to the incorporation of fruit pulp enhancing the bioactivity (Blassy *et al*., 2020). The rich content of polyphenol, flavonoids and other phytochemical content of *Prunus napaulensis* acted synergistically with the yoghurt matrix to improve the free radical scavenging activity (Rymbai *et al*., 2016; Shi *et al*., 2023). Emam and El-Nashar (2022) reported a significant improvement in antioxidant activity upon the addition of fruit extracts in yoghurts. The addition of pomegranate peel and honey to freeze-dried yoghurt has been reported to increase the phenolic content thereby increasing the antioxidant activity (Kennas *et al*., 2020). Durmus *et al*. (2021) reported the presence of anthocyanins such as cyanidin-3-glucosidase and cyanidin-3-rutinoside in black mulberry fortified yoghurt. The addition of guava fruit pulp, persimmon (*Diospyros kaki* L.) and mango (*Mangifera indica* L.) in functional yoghurt have been reported to significantly increase the radical scavenging activity due to the high phenolic and flavonoid content (Osman *et al*., 2020). Similarly the inclusion of grape juice

Table 1: Phytochemical content of fruit yoghurt with *Prunus napaulensis.*

Phytochemical	Control yoghurt	Fruit yoghurt
Total alkaloids (mg AE/g)	0.08±0.01	0.4±0.07*
Total flavonoids (mg QE/g)	0.56±0.02	2.68±0.1*
Total phenolics (mg GAE/g)	1.74±0.06	7.35±0.34*

Values are expressed as mean±SD (n=3). *Significantly different from control yoghurt ($p<0.05$) based on independent t-test. AE: Atropine equivalent, QE: Quercetin equivalent and GAE: Gallic acid equivalent.

with grape skin flour and grape seeds resulted in increased radical scavenging capacity and ferric iron-reducing power (Karnopp *et al.*, 2017).

Antidiabetic activity

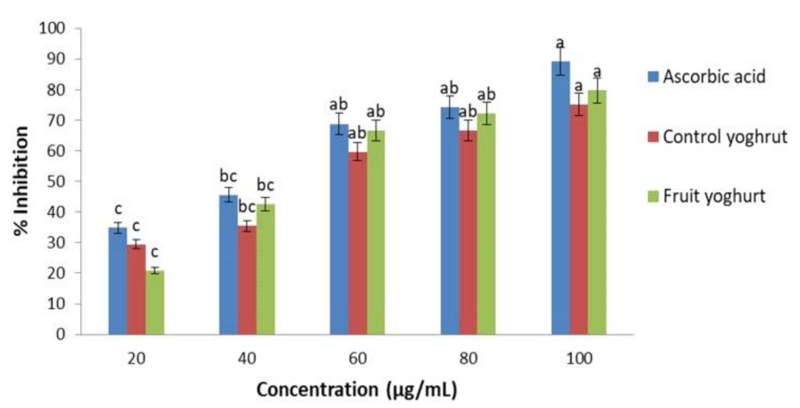
Antidiabetic activity of yoghurt samples expressed by α-amylase and α-glucosidase inhibitory activities are illustrated in Fig 2a and 2b respectively. The fruit yoghurt exhibited significantly higher α-amylase inhibitory activity as well as α-glucosidase inhibitory activity ($p<0.05$) as compared control yoghurt but lower activity than the standard, acarbose.

The higher antidiabetic activity of fruit yoghurt could be attributed to bioactive compounds such as polyphenols, flavonoids and other secondary metabolites which are known for their anti-diabetic properties (Alam *et al.*, 2022; Shi *et al.*, 2023; Swer *et al.*, 2016). Shori (2020) reported the antidiabetic properties of polyphenol rich foods in yoghurt which corroborates the role of *Prunus napaulensis* enhancing the bioactivity of yoghurt. In the present study, Antidiabetic activity of fruit yoghurt with *Prunus napaulensis* could be added to the previous studies that emphasize the functional yoghurt in the management of diabetes mellitus. Ni *et al.* (2018) reported the antidiabetic potential of yoghurt formulated with salal berry (*Gaultheria shallon*) and blackcurrant (*Ribes nigrum*) by inhibiting α-amylase, α-glucosidase and dipeptidyl peptidase IV enzymes involved in the blood glucose regulation. Yoghurt fortified with blackcurrant exhibited higher α-glucosidase inhibitory activity. Toledo *et al.* (2018) reports the increase of soluble fiber and mineral content thereby reducing the risk of diabetes in yoghurt incorporated with passion fruit peel and seed flour. Yoghurt enriched with elderberry juice exhibited significant inhibition of α-amylase and α-glucosidase enzymes (Cais-Sokoliñska and Walkowiak-Tomczak, 2021)

Anti-inflammatory activity

Anti-inflammatory activity of control yoghurt and fruit yoghurt was observed to be dose dependent with reference to the aspirin as evaluated by protease inhibition and protein denaturation assays (Fig 3). In protein denaturation assay, aspirin, control yoghurt and fruit yoghurt exhibited 24.91, 17.5 and 18.45 per cent of inhibition at 20 µg/mL respectively. Similarly, 72.16 per cent inhibition in fruit yoghurt was significantly higher than 68.5 per cent in control yoghurt, while aspirin exhibited 69.23 per cent of inhibition at 100 µg/mL ($p<0.05$). Protease inhibition was also observed to be significantly higher ($p<0.05$) in fruit yoghurt than control yoghurt with protease inhibition of 10, 11.63 and 17.43 per cent at 20 µg/mL for control yoghurt, fruit yoghurt and aspirin respectively. At 100 µg/mL, the protease inhibition of the fruit yoghurt (74.3 per cent) was significantly higher than inhibition of control yoghurt (68.4 per cent) while the standard aspirin exhibited 87.69 per cent of protease inhibition ($p<0.05$).

As an anti-inflammatory drug, aspirin, a non-steroidal drug (NSAID) inhibits protease and protein denaturation, which are associated with mechanism of inflammatory pathways (Obanla *et al.*, 2016). Similar anti-inflammatory effect could be attributed to bioactive peptides, fermentation process, probiotic cultures (Kashung and Karuthapandian, 2025; Paul *et al.*, 2023; Rekha *et al.*, 2021; Yahfoufi *et al.*, 2018) in control yoghurt and enhanced anti-inflammatory effect in fruit yoghurt could be linked to flavonoids, polyphenolic compounds of *Prunus napaulensis* incorporated in yoghurt with supportive studies (Hussain *et al.*, 2021; Kashyap *et al.*, 2022; Politis and Theodorou, 2016; Yahfoufi *et al.*, 2018). Pei *et al.* (2017) reported similar potential of yoghurt in the reduction of inflammatory cytokines (TNK-α and IL-6). Noni juice fortified yoghurt significantly increased the anti-inflammatory cytokine IL-10 in mice with ulcerative colitis and decreased pro-inflammatory cytokines (IL-6 and IF- γ) (Kwon *et al.*, 2021). Yoghurt supplemented with mulberry pomace exhibited comparable polyphenol-mediated anti-inflammatory effects (Du *et al.*, 2022), strawberry enriched yoghurt demonstrated the bioactive



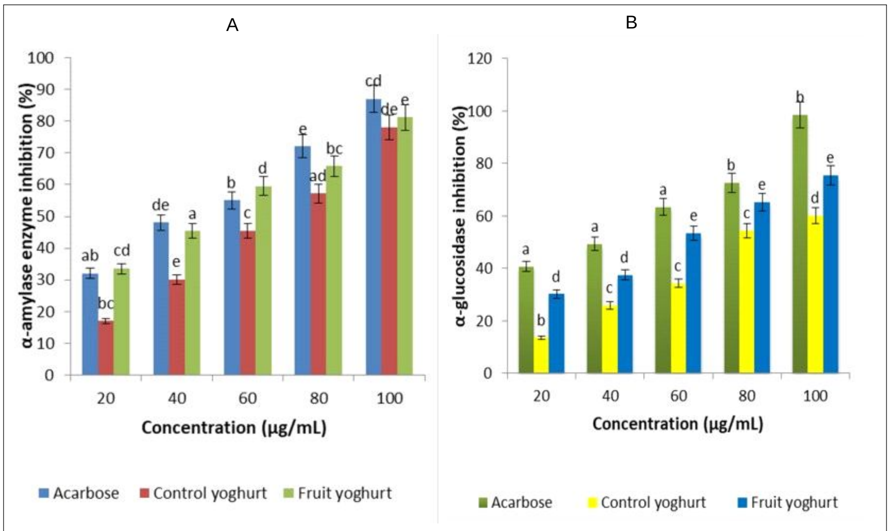
Data in triplicates are expressed as mean±SD. Bars with different alphabets indicates significant difference in activity among samples by one way ANOVA followed by Tukey's post-hoc test ($p<0.05$).

Fig 1: DPPH antioxidant activity of ascorbic acid (Standard), control yoghurt and fruit yoghrut.

potential of polyphenol through gastrointestinal digestion with increased radical scavenging activity at the intestinal level (Oliveira and Pintado, 2015). Limited studies have assessed the anti-inflammatory potential of fruit based yoghurts using both protein denaturation and protease inhibition assays, highlighting the relevance of this dual approach a comprehensive assessment.

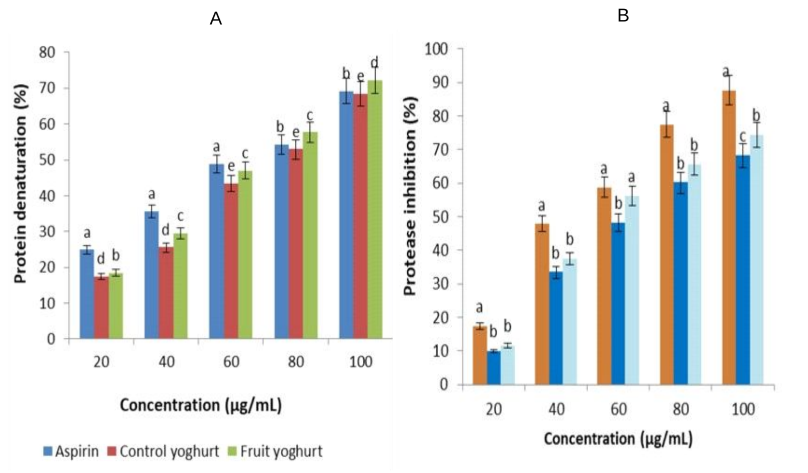
Antimicrobial activity

Antibacterial activity was observed in terms of zone of inhibition of growth of infective bacteria in control and fruit yoghurts as compared to Gentamycin as a positive control at 20, 40 and 60 µL/mL as shown in Fig 4 and Plate 2 respectively. *Bacillus cereus* was observed with a significant higher zone of inhibition ($p<0.05$) in fruit yoghurt (28.6±0.5 mm) than in control yoghurt (16.6±1.1 mm) at 60 µg/mL, while zone of inhibition was not observed in both control and fruit yoghurt at 20 µg/mL and 40 µg/mL. *Staphylococcus aureus* was observed with a significantly higher zone of inhibition at 20 µg/mL (15.3±0.5 mm), 40 µL/mL (19.7±0.4 mm) and 60 µL/mL (20±0.5 mm) of fruit yoghurt whereas control yoghurt exhibited zone of inhibition (17±2 mm) only at



Data in triplicates are expressed as mean±SD. Bars with different alphabets indicates significant differences in activity among samples by one way ANOVA followed by Tukey's post-hoc test ($p<0.05$).

Fig 2: Antidiabetic activity of acarbose (Standard), control yoghurt and fruit yoghurt against α-amylase enzyme (A) and α-glucosidase enzyme (B).

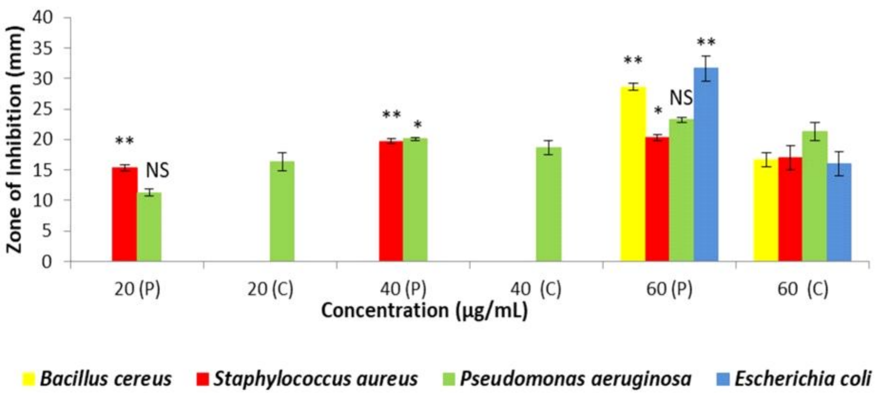


Data are expressed as mean±SD. a, b, c indicate statistically significant differences between groups based on Tukey's post-hoc test ($p<0.05$).

Fig 3: Anti-inflammatory activity of Aspirin (standard), control yoghurt and fruit yoghurt in Protein denaturation assay (A) and Protease inhibition assay (B).

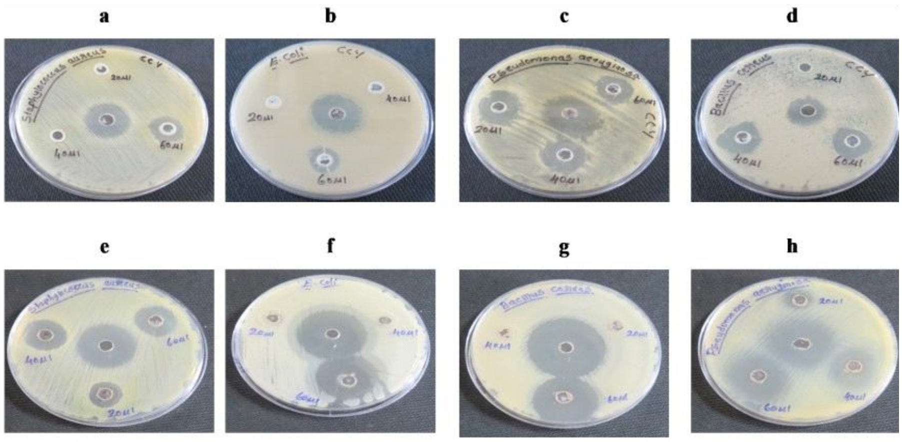
60 µg/mL (p<0.05). At 20, 40 and 60 µg/mL of samples, zone of inhibition of *Pseudomonas aureginosa* was 11.3±0.5mm, 20.1±0.2 mm and 23.2±0.4 mm respectively in fruit yoghurt whereas 16.3±1.5 mm, 18.6±1.1 mm and 21±1.5 mm respectively in control yoghurt (p<0.05). Similarly, only at 60 µg/mL of sample, higher zone of inhibition (p<0.05) for *Escherichia coli* was observed in fruit yoghurt (31.6±2 mm) than in control yoghurt (16±2 mm).

The antimicrobial activity of control yoghurt is often attributed to the lactic acid bacteria, organic acids and the bioactive peptides produced during fermentation (Dimitrova-Dicheva *et al.,* 2021; Nuralifah *et al.,* 2022; Taha *et al.*, 2017). Suriyaprom *et al.* (2022) suggest that phenolic compounds from fruits are known to interfere with the bacterial proteins and enzymes leading to the inhibition of bacterial growth. Phenolic acids and tannins are known to inhibit the growth of Gram's negative bacteria (Coppo and Marchese, 2014). The synergistic effects of bioactive compounds such as polyphenols and flavonoids may increase the inhibition zones as reported (Blassy *et al.*, 2020; Dimitrellou *et al.,* 2020; Szołtysik *et al.*, 2021). Recent studies have reported the antimicrobial potential of fruit enriched yoghurts. Yoghurt supplemented with *Siraitia grosvenrii* fruit extract demonstrated antibacterial activities against *Escherichia coli, Salmonella typhimurium* and *Listeria monocytogenes* (Abdel-Hamid *et al.,* 2020), yoghurt incorporated with plant extracts exhibited significant activity against *Escherichia coli, Bacillus cereus, Staphylococcus aureus* and *Candida albicans* (Bayram *et al.*, 2024). Pineapple incorporated yoghurt demonstrated significant antimicrobial activity against *Escherichia coli* (Auli *et al*., 2025), similarly yoghurt enriched with pomegranate



Data are expressed as mean±SD.* Significant (p<0.05), **Highly significant (p<0.01) and NS= not significant, based on independent t-test.

Fig 4: Antimicrobial activity of control yoghurt (C) and fruit yoghurt (P) in varying concentrations against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli.*



Representative images show zones of inhibition produced by samples at concentration of 20, 40 and 60 µg/mL. Clear zones indicate microbial growth inhibition, demonstrating the comparative antimicrobial efficacy of the samples.

Plate 2: Antimicrobial activity of the control yoghurt (a, b, c, d) and fruit yoghurt with *Prunus napaulensis* (e, f, g, h) *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli*.

exhibited significant antimicrobial activity storage against yeast, mold, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Zahed and Kenari, 2025).

Although this present study focused on the *in vitro* bioactivity evaluation, sensory attributes, physico-chemical parameters and shelf life stability of *Prunus napaulensis* yoghurt have been previously reported (Kashung *et al*., 2025) exhibiting an acceptable overall acceptability score and confirmed product stability throughout 21 day storage. In vivo validation through animal model and clinical trials are required to confirm the bioavailability and physiological relevance of these functional effects.

CONCLUSION

The present study provides an insight on functional incorporation of pulp of *Prunus napaulensis* (7 per cent (w/v) in to yoghurt matrix balancing the fruit acidity overcoming curdling in yoghurt gel formation with consumer acceptability as well as functionality for human health benefits. The incorporation of *Prunus napaulensis* fruit in yoghurt significantly increased the phytochemical composition compared to control yoghurt. The fruit yoghurt with *Prunus napaulensis* was proved for the enhancement in antioxidant activity (DPPH and FRAP assays), antidiabetic activity (α-amylase and α-glucosidase enzymes), anti-inflammatory activity (protein denaturation inhibition and protease inhibition activity) along with antimicrobial activity against inhibition of growth zone of *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli.* It could be attributed to the solubility and bioavailability of phenols, flavonoids, alkaloids and organic acid of *Prunus napaulensis* to conjugate with bioactive peptides, probiotics and lactic acid in the combined fat and aqueous media of yoghurt matrix. Hence the formulation of fruit yoghurt with *Prunus napaulensis* would be an appropriate suitable functional dairy product as a functional approach in the mitigation of public health burden due to chronic diseases in the present trend.

This study contributes valuable insights into the valorization of underutilized indigenous fruits from Northeast India supporting both nutritional enhancement and sustainable food system development. Further studies focusing on the isolation and characterization of specific bioactive compounds and peptides in the fruit yoghurt in clinical validation and in vivo assessments can further substantiate its application in functional food development.

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Conflict of interest

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