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Exploration and Purification of Secondary Metabolites from *Lactobacillus acidophilus* and Their Potential as a Bio-preservative

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The natural tendency of many food items to spoil requires the use of natural preservatives to extend shelf life while maintaining their original qualities, such as texture and flavour. Consequently, there's a rising demand for preservatives derived from organic sources. This research focused on producing a bioactive compound with a low molecular weight using *Lactobacillus acidophilus* (MTCC 10307). The study investigated how different production mediums, temperatures, and pH levels influenced the synthesis process. With optimized conditions, the molecule was synthesized, extracted, purified, and characterized. UV analysis detected an absorption peak characteristic of a

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proteinaceous substance in the extracted material. The isolated compound was then tested for its antibacterial activity against foodborne pathogens. By utilizing the isolated component as a bio-preservative in various types of juices, its effect on shelf life was assessed. One advantage of a low molecular weight compound is its ability to quickly penetrate cell walls and combat bacteria responsible for food spoilage.

Keywords: Lactic acid; Lactobacillus acidophilus; bio preservation; antibacterial protein; food preservation.

1. INTRODUCTION

In recent years, there has been a noticeable surge in the demand for safe and natural food preservatives, driven by consumer preferences for healthier and more sustainable food choices. This trend has sparked a heightened interest in harnessing secondary metabolites produced by microorganisms, particularly probiotic bacteria, as potential bio-preservatives in the food industry. Among these probiotics, *Lactobacillus acidophilus* (*L. acidophilus*) stands out as a compelling candidate due to its well-established safety record, probiotic qualities, and ability to produce a diverse array of secondary metabolites with antimicrobial properties [1].

L. acidophilus (MTCC 10307) is a thoroughly studied strain known for its probiotic effectiveness and resilience in harsh environments, such as the acidic conditions of the human gastrointestinal tract. Extensively researched for its health-enhancing properties, this strain has shown efficacy in promoting gut health, strengthening immune function, and alleviating gastrointestinal disorders. However, recent investigations have also shifted towards exploring its potential applications in the food industry, particularly as a source of natural preservatives [2].

Secondary metabolites are organic compounds produced by microorganisms that aren't directly involved in growth or reproduction but often provide survival advantages, such as protection against competing microorganisms. These metabolites exhibit diverse biological activities, including antimicrobial, antioxidant, and antifungal properties, making them valuable tools for food preservation. *Lactobacillus acidophilus* is known for its production of various secondary metabolites, including bacteriocins, organic acids, exopolysaccharides, and biosurfactants, all of which possess potent antimicrobial properties against a wide array of foodborne pathogens and spoilage microorganisms [3].

Bacteriocins, for example, are small antimicrobial peptides produced by lactic acid bacteria like *L. acidophilus*. They function by inhibiting the growth of closely related bacteria through mechanisms such as disrupting cell membranes or interfering with essential cellular processes. These peptides have garnered significant attention as natural alternatives to chemical preservatives due to their broad-spectrum antimicrobial activity, heat stability, and safety for consumption. Moreover, bacteriocins from *Lactobacillus acidophilus* have shown synergistic effects when combined with other antimicrobial agents, thereby enhancing their potential as biopreservatives in food [4].

Organic acids, such as lactic acid and acetic acid, are metabolic byproducts produced by microorganisms during fermentation, contributing to the acidic environment characteristic of fermented foods. These acids not only decrease the pH of the food matrix, inhibiting the growth of spoilage microorganisms, but also exert direct antimicrobial effects against a wide spectrum of pathogens. *L. acidophilus* is renowned for its production of significant quantities of lactic acid, which not only impart the distinctive flavor and texture to fermented dairy products but also play a crucial role in extending their shelf life [5].

Exopolysaccharides (EPS) are complex carbohydrate polymers secreted by certain bacteria, including *Lactobacillus acidophilus*, forming a protective matrix around microbial cells, thereby enhancing their survival in adverse conditions. Besides their involvement in bacterial encapsulation and biofilm formation, EPS have exhibited antimicrobial activities against various foodborne pathogens, making them promising candidates for use as bio-preservatives in food [6].

Bio-surfactants are surface-active molecules synthesized by microorganisms, capable of reducing surface tension and enhancing the solubility of hydrophobic compounds. These molecules exhibit antimicrobial properties by

disrupting the cell membranes of target microorganisms. *Lactobacillus acidophilus* has been reported to produce bio-surfactants with potential applications in food preservation, including inhibiting biofilm formation and controlling foodborne pathogens [7].

In summary, *L. acidophilus* (MTCC 10307) emerges as a promising source of natural bio-preservatives for the food industry. This is due to its ability to produce a diverse range of secondary metabolites with potent antimicrobial activities. By investigating the production and purification of these metabolites, researchers can further elucidate their mechanisms of action and evaluate their efficacy in preserving various food products. Moreover, the development of innovative bio-preservation strategies based on *Lactobacillus acidophilus* metabolites holds promise for producing safer, healthier, and more sustainable food items, meeting the growing consumer demand for natural and minimally processed foods.

2. MATERIALS AND METHODS

2.1 *Lactobacillus acidophilus* (*L. acidophilus*) Inoculation

The starter culture of *L. acidophilus* (MTCC 10307) was inoculated in the De Man, Rogosa and Sharpe broth (MRS broth) medium and incubated at 37°C for 24 hours. The stored cultures were further used for production of compounds.

2.2 Biomass Estimation

In the pre-weighed Eppendorf tubes, 1 ml of culture was taken and pellet was collected by centrifugation at 5000 rpm for 5 minutes. The supernatant was discarded fully and the pellet was weighed to identify the biomass of the two samples [8]. Formula used is:

Biomass estimation = Final weight of the tube - Initial weight of the tube

2.3 Antibacterial Activity of Compound Produced

The antibacterial activity of *L. acidophilus* was assessed against pathogenic organisms following the method outlined by Todorov and Dicks [9]. Initially, a sample of the produced compound was collected in an Eppendorf tube. Subsequently, 30 ml of Muller Hinton agar (MHA) was prepared and poured into two Petri plates, allowing it to solidify. Using a well cutter, three wells were created in each agar plate. Then, 20 µl and 40 µl of the produced compound were added to the first two wells, while 20 µl of distilled water (D.W) was added to the third well, in both agar plates containing actively growing cells of the test organisms, *E. coli* and *S. aureus*. Finally, an antibiotic disk (Chloramphenicol) was placed in the corner of each plate using forceps. The plates were then incubated for 24 hours at 37°C in an incubator, followed by the measurement of the zone of inhibition in each case [9].

2.4 Purification of the Compound using Ammonium Sulphate Precipitation

L. acidophilus was inoculated into MRS broth in a conical flask and then incubated at 37°C for 24 hours. Afterward, the supernatant of the culture was obtained by centrifuging at 5000 rpm for 5 minutes. The supernatant was then transferred from the Eppendorf tube to two screw-cap bottles. The first bottle was designated as the control (crude), while 6g of ammonium sulphate was added to the second bottle for precipitation, followed by incubation at 4°C for 2 days. Subsequently, the broth containing ammonium sulphate was transferred into two Eppendorf tubes and centrifuged at 5000 rpm for 3 minutes. This centrifugation process was repeated until the broth was depleted. The supernatant was discarded, and 2 ml of phosphate buffer was added to each tube, followed by mixing with the pellet. The mixture was then transferred back into the screw-cap bottle, resulting in a total volume of 7 ml in each bottle. Finally, the optical density (OD) of this mixture was measured at 280 nm, along with the control.

Furthermore, using a semipermeable membrane (50kDa Hi Media Mumbai), dialysis of the mixture obtained in phosphate buffer was performed, and it was kept for 2 days at 4°C [10].

2.5 Column Purification

A column packed with Sephadex 50 gel is utilized, with distilled water (DW) used for washing the column five times, followed by phosphate buffer used twice to further rinse the column. The column measures 160cm in length. Subsequently, the 2 ml sample obtained from dialysis using a semipermeable membrane is combined with 3 ml of phosphate buffer to yield a total volume of 5 ml. This solution is then loaded into a burette and carefully transferred drop by

drop into Eppendorf tubes, aiming for approximately 1 ml of solution in each tube. The loaded Eppendorf tubes are then placed in a refrigerator at 4ºC for a duration of 2 days. Finally, the optical density (OD) of the solutions within the different Eppendorf tubes is measured at 280 nm [11].

2.6 Antibacterial Activity of Sample after Column Purification

The antibacterial activity of the bio-preservative sample with the two highest optical densities (OD) following column purification was evaluated against pathogenic organisms, employing the method outlined by Todorov and Dicks [12]. Initially, samples of the bio-preservative compound were collected in Eppendorf tubes. Subsequently, 45 ml of MH agar was prepared and poured into three Petri plates, allowing it to solidify. Four wells were then created in each plate using a well cutter. Next, 20 µl of the control and the two samples with the highest OD were added to the first three wells, while 20 µl of distilled water was added to the fourth well, in all three agar plates seeded with actively growing cells of the test organisms, *E. coli, S. aureus*, and *K. pneumoniae*. Finally, antibiotic CTX30 (Cefotaxime) disks were placed in the centre of each plate using forceps. Subsequently, all plates were incubated for 48 hours at 37°C in an incubator, followed by the measurement of the zone of inhibition in each case.

2.7 Scale Up Production of Compound

Prepare 30 ml of MRS broth in C.F and add *L. acidophilus* culture to it, then incubate it for 48 hrs at 40ºC [13].

2.8 Partial Purification of Compound

The partial purification of the bio-preservative compound produced from *L. acidophilus* involved utilizing ammonium sulphate precipitation to isolate the bio-preservative from the mixture for subsequent studies. Initially, *L. acidophilus* was inoculated into MRS broth contained in a conical flask and then incubated at 37ºC for 2 days. Following incubation, the supernatant of the culture was obtained by centrifuging at 6000 rpm for 5 minutes. Subsequently, the supernatant from the entire Eppendorf tube was transferred into two screw-cap bottles, each with a volume of 15 ml. These screw-cap bottles were then labelled as sample 1 and sample 2 [13].

2.9 Ammonium Sulphate Precipitation

In sample 1, 6g of ammonium sulphate was added to the cork screw cap. The sample was then incubated for 24 hours at 4ºC to facilitate precipitation. After incubation, the sample was transferred into two Eppendorf tubes and centrifuged at 6000 rpm for 5 minutes. This centrifugation process was repeated until the entire sample was processed. The supernatant in the two Eppendorf tubes was discarded, and 0.5 ml (500 µl) of phosphate buffer was added to each tube. The solution was thoroughly mixed. Finally, the resulting solution was used for SDS-PAGE analysis [14].

2.10 Aqueous Two Phase Extraction using Chloroform and Ethyl Acetate

To sample 2 in the cork screw cap bottles, 5 ml of chloroform and 5 ml of ethyl acetate were added, and the mixture was thoroughly mixed. The sample was then incubated for 24 hours at 4ºC. After incubation, the sample was retrieved and the upper phase formed was carefully transferred into another cork screw cap bottle using a micropipette. Subsequently, the optical density (O.D) at 280 nm was measured using a UV-visible spectrometer for the identification of the protein [14].

2.11 Characterization

2.11.1 UV- VIS study

To determine the maximum absorbance of the extracted peptide diluted protein samples, analysis was conducted on an ELICO SL 159 UV-VIS spectrophotometer within the wavelength range of 220 nm to 400 nm. The data were collected using UV software, and peak values were identified from the graphical representation generated by the spectrophotometer.

2.11.2 SDS page

The stacking and separating gel were loaded and maintained at 50V. Subsequently, the gel was immersed in a staining dye for 12 hours. Finally, the protein bands were visualized, allowing for the identification of the molecular weight of the compound [10].

2.11.3 FTIR (Fourier- transform infrared spectroscopy)

An FTIR study was conducted to ascertain the functional groups present in the sample. The absorbance in the FT-IR spectra of the samples was documented using a spectrometer, with data collected within a scanning range of 400-4000 cm^-1 [10].

2.11.4 Thin layer chromatography

To perform chromatography using MERCK silica gel coated paper, begin by drawing a line 1 cm away from one end of the paper. On the opposite end, mark 'CH' (Chloroform) and 'AC' (Ammonium sulphate crude). Make two points on the line drawn near one end. Using a toothpick, add ammonium precipitate from the semi-permeable membrane and the chloroform supernatant on the points corresponding to the markings on the other side, repeating this process at regular intervals of 2 minutes for at least 25 times.

Next, immerse the paper in a beaker containing the mobile phase, which consists of chloroform and methanol in a 10:1 ratio. Allow the mobile phase to ascend across the stationary phase on the paper. Once the mobile phase has traversed, remove the paper and let it dry. Place the dried strip over a hot plate after adding ninhydrin to visualize the compounds. Calculate the Rf value for each compound.

Afterwards, scrape out the compounds separated out in both the chloroform sample and the ammonium crude sample using a toothpick, and store them separately in PCR tubes. These compounds can later be used for antimicrobial activity studies of the separated bio-preservative compound [10].

2.11.5 Antibacterial activity of bio preservative compound

To test the antibacterial activity of the bio-preservative samples obtained from chloroform scrap and ammonium crude scrap in the PCR tubes obtained after thin layer chromatography (TLC), the method described by Todorov and Dicks (2004) was followed.

First, prepare 60 ml of MH agar and pour it into four Petri plates, allowing it to solidify. Using a well cutter, create three wells in each plate. Add the chloroform sample and ammonium crude sample into the first two wells of each plate, and add 20 µl of phosphate buffer solution into the third well.

Next, inoculate the agar plates with actively growing cells of the test organisms *E. coli*, S. *aureus, S. typhi*, and *B. cereus*. Place antibiotic CTX30 (Cefotaxime) disks in the corner of each plate using forceps.

Incubate all the plates for 48 hours at 37ºC in an incubator. After incubation, calculate the zone of inhibition in each case, indicating the effectiveness of the bio-preservative samples against the pathogenic organisms [9].

2.11.6 Application in food

Applications in fruit juice: The application in fruit juice is performed using apple and sapodilla juice. Six sterilised screw cap conical flasks and 100 ml distilled water were taken. Take the fruit samples (apple and sapodilla) and then cut it into small pieces. Then grind these pieces in distilled water separately using mortar and pestle in laminar air flow (ALF) to make it into juice. Transfer the apple juice in 3 cork screw cap flasks and likewise transfer the sapodilla juice into 3 cork screw flasks in LAF [15].

Apple juice samples: The apple juice contained in the 3 cork screw flasks, are taken such that the first flask sample is taken as control, to the second flask sample 100 µl of sodium benzoate is added and to the third flask add 100 µl of bio preservative compound is added. Keep it for incubation at 37ºC. Observe the samples in flasks in 5th and 10th day.

Sapodilla juice samples: The sapodilla juice contained in the 3 cork screw flasks, are taken such that the first flask sample is taken as control, to the second flask sample 100 µl of sodium benzoate is added and to the third flask add 100 µl of bio preservative compound is added. Keep it for incubation at 37ºC. Observe the samples in flasks in the 7th day [15].

3. RESULTS

3.1 Antibacterial Activity of Compound Produced

Two different bacterial species, *E. coli* and *S. aureus*, were used to identify the antibacterial activity of the bio-preservative compound. Swabs of these bacterial species were streaked onto plates containing MH agar. Two different volumes of the supernatant (20 µl and 40 µl) were added to the plates, and after incubation at 37ºC for 24 hours, zones of inhibition were observed around the areas where the supernatant was added. Additionally, the antibiotic Chloramphenicol (C30) displayed clear zones of inhibition in both cases, as illustrated in Fig. 1 and Table 1.

(a) **(b)**

Fig. 1. Antibacterial activity of compound produced A- *E. coli*; B- *S. aureus*

Table 1. Zone of inhibition of antibacterial activity of compound produced

S. No	Name of pathogen	Volume of supernatant (20 µl)	Volume of supernatant (40 µl)	Disk (C30)
1)	*E. coli*	8mm	11mm	9mm
2)	*S. aureus*	4mm	6mm	10mm

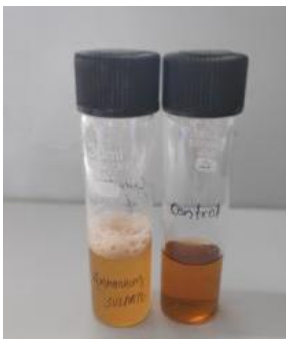


Fig. 2. The media precipitated with ammonium sulphate and the control after 24 hrs of incubation

Table 2. Optical density of the Medium precipitated with ammonium sulphate and the control

S. No	Sample	OD at 280 nm
1)	Medium precipitated with ammonium sulphate	0.080
2)	Control	0.046

Purification of compound using ammonium sulphate precipitation: After the incubation for 24 hrs at 4ºC, there is cloudy formation with bubbles at the top in the occurred in the medium precipitated with ammonium sulphate, as shown in Fig. 2.

The OD of the medium is taken after the pellet obtained by centrifugation is added with phosphate buffer solution which gives (as shown in Table 2);

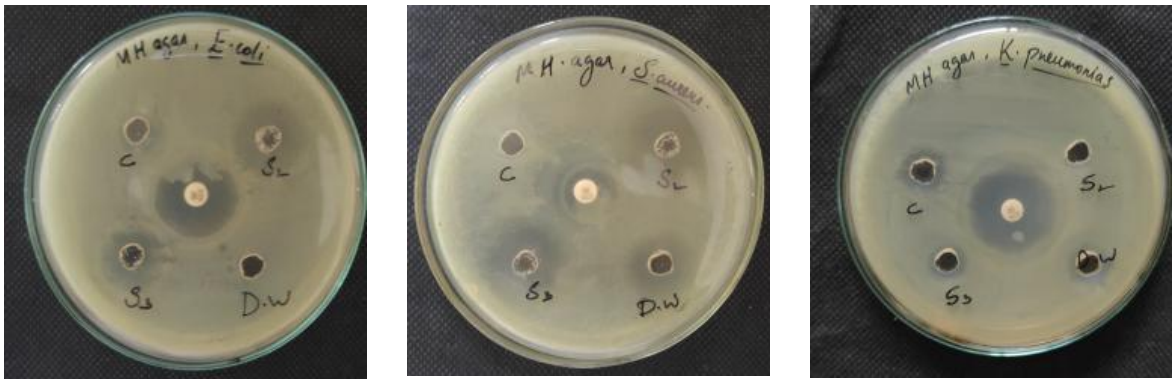
The OD of the medium which is precipitated with ammonium sulphate has a higher than the OD value than that of the control, which means that medium precipitated is purified.

3.2 Column Purification

After the column purification the transferred solution kept in the Eppendorf tubes incubated at 4ºC for 2 days (as shown in Fig. 3). Then the OD of the samples in the Eppendorf tubes taken at 280nm (as shown in Table 3).

(a) (b)

Fig. 3. a - The column purification using the sephadex 50 gel; b - drop by drop transfer into the Eppendorf tubes



(a) (b) (c)

Fig. 4. Antibacterial activity of the compound after column purification a - *E. coli*; b - *S. aureus*; c - *K. pneumonias*

Table 3. The values of the OD of samples purified by column purification at 280 nm

Sample	OD AT 280 NM
S1	0.018
S2	0.074
S3	0.082
S4	0.014
S5	0.011

Table 4. The Zone of the inhibition of the compound after column purification

S. No	Name of pathogen	Zone of inhibition (Control)	Zone of inhibition (S2)	Zone of inhibition (S3)	Zone of inhibition (Disk CTX 30)
1)	*E. coli*	1mm	8mm	6mm	10mm
2)	*S. aureus*	1mm	14mm	8mm	6mm
3)	*K. pneumonias*	3mm	1mm	2mm	9mm

Here the 2 samples with the highest OD are taken for the analysis of antimicrobial activity of the compound after column purification.

3.3 Antibacterial Activity of Compound after Column Purification

Three different bacterial species, *E. coli, S. aureus*, and *K. pneumoniae*, were used to identify the antibacterial activity of the bio-preservative compound after column purification. Swabs of these bacterial species were streaked onto plates containing MH agar. In each plate, 20 µl of the control was added to one well, and the purified compound after column purification with the highest 2 optical densities (OD) (S2, S3) was added to other wells. After incubation at 37ºC for 24 hours, zones of inhibition were observed

around the areas where the purified compound was added. Additionally, the antibiotic Cefixime (CTX30) displayed clear zones of inhibition in all three cases, as illustrated in Fig. 4 and Table 4.

3.4 Partial Purification of Compound

3.4.1 Ammonium sulphate precipitation

The ammonium precipitation process was conducted at 70% saturation and incubated overnight at 4ºC. Following incubation, the mixture was centrifuged to obtain the precipitate, which contains the bio-preservative compound. The precipitate obtained was dissolved using phosphate buffer and thoroughly mixed to obtain a cell-free extract. This extract, containing the partially purified secondary metabolite, was then utilized for SDS-PAGE analysis, as depicted in Fig. 5.

3.5 Aqueous Two-Phase Extraction using Chloroform and Ethyl Acetate

In the aqueous two-phase extraction process utilizing chloroform and ethyl acetate, the upper phase, which contains the extracted secondary metabolite, is separated and transferred into another cork screw bottle using a micropipette. The final solution, now containing the extracted secondary metabolite, is then utilized for UV-VIS spectrometry study, as depicted in Fig. 6.

3.6 Characterization

3.6.1 UV-VIS spectroscopy

The peak values observed for the maximum absorbance of the extracted compound is checked and it was found at 280 nm, as shown in the Fig. 7.

3.6.2 SDS page

After loading the sample into the well, the SDS gel was allowed to separate the protein. Following the electrophoresis run, the gel containing the extracted protein was visualized under a UV trans-illuminator. The molecular weight of the protein was determined by comparing it to protein markers loaded alongside, as illustrated in Fig. 8. The molecular weight of the protein was found to be 19 kDa.

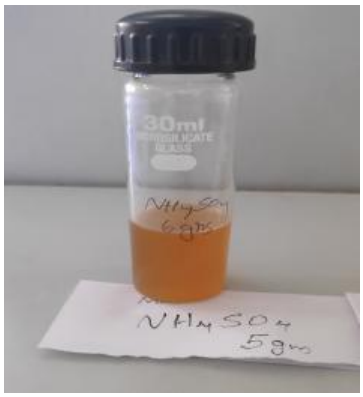


Fig. 5. The partial purification using ammonium sulphate precipitation

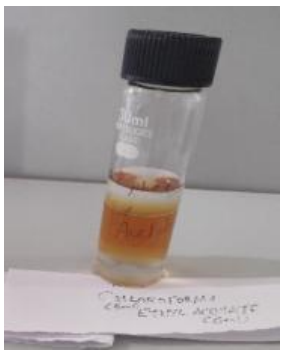


Fig. 6. The extraction of compound using aqueous two phase separation

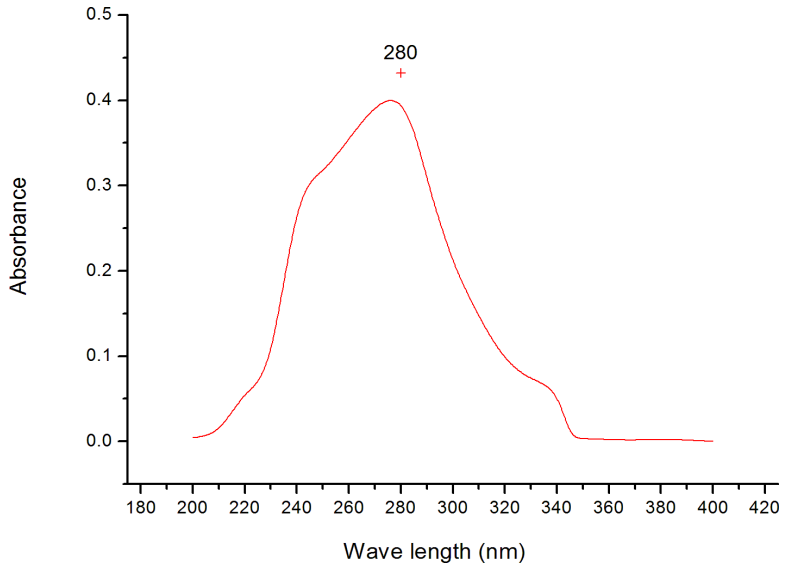


Fig. 7. The absorbance of the extracted compound is studied using UV-VIS spectroscopy

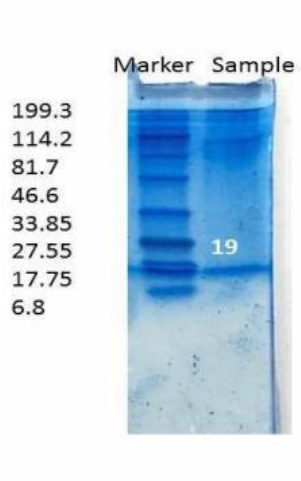


Fig. 8. SDS - PAGE for the extracted compound

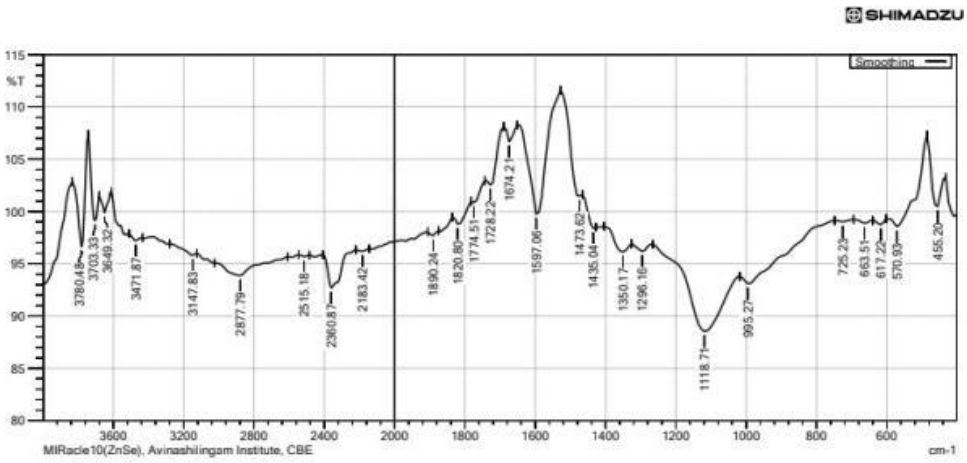


Fig. 9. FTIR study on the compound extracted

Table 5. Functional groups of FTIR study for the protein extracted

Peak values	Type of vibration Causing ir adsorption	Functional group
455.20	-	-
570.93	-	-
617.22	-	-
663.51	-	-
725.23	-	-
995.27	-	-
1118.71	C-O Stretch	Ether
1296.16	N=O Bend	Nitro Bend
1350.17	N=O Bend	Nitro Bend
1435.04	H-C-H Bend	Alkanes
1473.62	H-C-H Bend	Alkanes
1597.06	N-H Bend	Amides
1674.21	C=O Stretch	Esters
1728.22	C=O Stretch	Esters
1774.51	C=O Stretch	Esters
1820.80	-	-
1890.24	-	-
2183.42	C=C Stretch	Alkynes
2360.87	C=N Stretch	Nitriles

Table 6. RF value of chloroform compound and ammonium crude compound

S. No	Sample	Rf value
1)	Chloroform	0.121
2)	Ammonium Crude	0.067

(a) **(b)**

Fig. 10. TLC on the protein; a- TLC experiment; b - The silica coated paper with the compounds separated after dried out

3.6.3 FTIR

The absorbance FT - IR spectra of the protein was documented using a spectrometer. The samples collected within a scanning were collected within a scanning range of 400-4000 cm-1 were shown in Fig. 9 & Table 5.

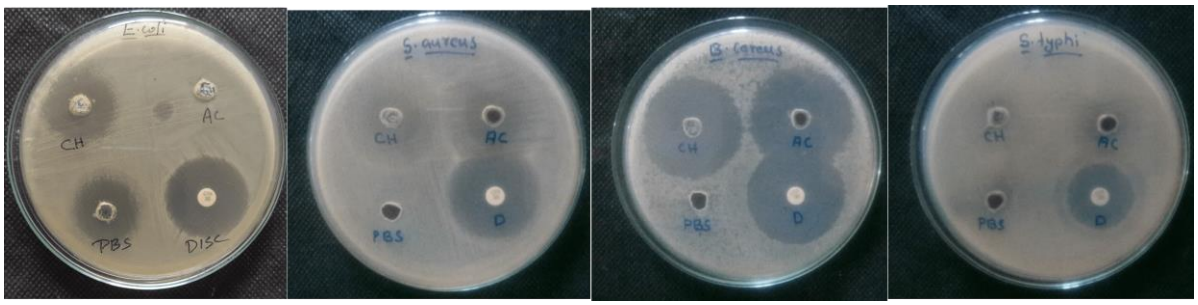
3.7 Thin Layer Chromatography (TLC)

The Rf (retention factor) value of the protein compound, crucial for identifying the compound, was calculated after applying ninhydrin to the silica gel coated paper. Subsequently, the compound separated was scraped out and stored in PCR tubes for further analysis to determine its antibacterial activity. The Rf values confirmed the presence of low molecular weight compounds, as illustrated in Fig. 10 and Table 6.

Rf value = Distance moved by solute/ Distance moved by solvent

3.8 Antibacterial Activity of the Bio-preservative Compound

E. coli, S. aureus, B. cereus, and *S. typhi* were swabbed onto plates containing MH agar. In



(a) (b) (c) (d)

Fig. 11. Antimicrobial activity of the bio-preservative compound; *a- E. coli; b- S. aureus; c- B. cereus; d- S. typhi*

(a) (b)

Fig. 12. The juice samples on the 7th day after added with preservatives; a - apple juice; b - sapodilla juice

Table 7. Zone of inhibition of the Bio preservative compound.

S. No	Name of pathogen	Zone of inhibition (CH)	Zone of inhibition (AC)	Zone of inhibition (PBS)	Zone of inhibition (Disk CTX 30)
1)	*E. coli*	10mm	3mm	9mm	13mm
2)	*S. aureus*	1mm	14mm	8mm	6mm
3)	*B. cereus*	14mm	14mm	Nil	15mm
4)	*S. typhi*	3mm	3mm	2mm	9mm

each plate, 20 µl of the scrapped chloroform compound (CH) was added to one well, 20 µl of the scrapped ammonium crude (AC) compound was added to the second well, and phosphate buffer solution (PBS) was added to the third well. After incubation at 37ºC for 24 hours, zones of inhibition were observed around the areas where the chloroform and ammonium crude compounds were added. Additionally, the antibiotic Cefixime (CTX30) displayed clear zones of inhibition in all four cases, as shown in Fig. 11.

3.9 Application in Fruit Juice

On the 7th day from the day of incubation, samples of the fruit juices, including apple and sapodilla juices, supplemented with the bio-preservative compound and the chemical preservative sodium benzoate, were observed. These observations are depicted in Fig. 12.

4. DISCUSSION

The utilization of bio-preservative chemicals derived from natural sources offers a promising approach to enhancing food safety and prolonging shelf life [16]. This study investigates the synthesis and characterization of a bio-preservative compound produced by *Lactobacillus acidophilus* under standard conditions. The discussion encompasses the synthesis process, purification techniques, characterization methods, and potential applications of the bio-preservative compound in

combating foodborne pathogens and improving food preservation.

The bio-preservative chemical was synthesized using Lactobacillus acidophilus under standard conditions, maintaining a medium pH of 7 and a temperature of 40ºC. These conditions were optimized to facilitate the production of the desired compound effectively. Following synthesis, the compound underwent purification using ammonium sulphate precipitation and aqueous two-phase separation techniques. These purification methods ensured the isolation of the bio-preservative compound from other cellular components, enhancing its purity and efficacy [17].

The properties of the bio-preservative compound were characterized using a range of analytical techniques, including UV-VIS spectroscopy, thin-layer chromatography (TLC), Fourier-transform infrared spectroscopy (FTIR), and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). UV-VIS spectroscopy enabled the determination of the compound's absorption spectra, providing valuable insights into its chemical structure and composition. TLC analysis allowed for the separation and visualization of different compounds present in the sample. FTIR spectroscopy elucidated the functional groups present in the compound, aiding in its identification and characterization [18]. Additionally, SDS-PAGE was employed to determine the molecular weight of the compound, revealing a low molecular weight of 19 kDa, indicative of a secondary metabolite [19].

The bio-preservative compound displayed notable antibacterial activity against a range of foodborne pathogens, as evidenced in fruit juice samples. Its effectiveness in combating foodborne infections underscores its potential applications in food preservation and safety [20]. Additionally, the compound's low molecular weight facilitates its penetration through cell walls, thereby enhancing its capability to inhibit bacterial growth and extend the shelf life of food products.

5. CONCLUSION

The bio-preservative chemical was synthesized using *Lactobacillus acidophilus* under standard conditions, with a medium pH of 7 and a temperature of 40ºC. The purification of the bio-preservative compound involved processes such as ammonium sulphate precipitation and aqueous two-phase separation. The compound's properties were determined using UV-VIS spectroscopy, TLC, and FTIR techniques. SDS PAGE analysis revealed the protein's molecular weight to be 19 kDa, indicating that the chemical is a low molecular weight secondary metabolite.

The low molecular weight of the secondary metabolite enables it to readily pass through cell walls, allowing it to effectively combat foodborne infections and extend the shelf life of various food products. The antibacterial activity of the bio-preservative molecule was assessed against several foodborne pathogens, including in fruit juice samples, where it demonstrated active efficacy against these pathogens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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