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Characterization And Evaluation Of Metabolites Using *Lactiplantibacillus plantarum* (OP535992.1) Isolated From Indigenous Cow Milk And Its Antimicrobial, Anti-cancerous Properties.

Vipin Krishna SL*, R Ragunathan, and Jesteena Johney.

Department of Biotechnology, Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore - 21, Tamil Nādu, India.

ABSTRACT

The use of *Lactobacillus* to improve the health of human is a current scenario, lactic acid bacteria are probiotic and produce wide range of metabolites that can be used as antimicrobial, anticancerous and as biopreservates. The present study was carried out to produce metabolites to use as antimicrobial and anticancer agent.

Keywords: *Lactiplantibacillus plantarum*, Probiotics, metabolites, antimicrobial, anticancer.

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Corresponding author

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INTRODUCTION

Lactic Acid Bacteria (LAB) are a group of Gram-positive, non-sporulating, anaerobic, or facultative aerobic cocci or rods, which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrates. These bacterias are the major components of the starters used in fermentation, especially for dairy products, and some of them are also natural components of the gastrointestinal microflora. *Lactobacillus* is one of the most important genera of lactic acid bacteria. These organisms produce various known compounds such as bacteriocin which can antagonize the growth of some pathogenic bacteria in foods (Abdelbasset, Djamila. 2008). Lactic acid bacteria are regarded as a major group of probiotic bacteria and have been used successfully to treat acute infantile diarrhoea and various types of illnesses (Buie and Gorbach, *et al*. 1995).

Many lactic acid bacteria species play a major role in the ripening process of cheese, especially to improve the consistency, aroma, and flavour of the milk products (Hannon *et al*., 2003). Probiotics, microorganisms that are useful to health when consumed. Milk and milk products are usually associated with probiotic bacteria, which provide supplements for the beneficial maintenance of the intestinal system (Tambekar and Bhutada *et al.*, 2010). The prime Lactic acid-producing bacterial (LAB) groups are gram-positive, catalase-negative organisms and they belong to genera *Lactobacillus, Bifidobacterium, Lactococcus*, and *Leuconostoc* (Leroy and De Vuyst, 2004). There has been increasing attention to the use of diverse strains of lactic acid bacteria as probiotics, mainly *Lactobacilli* and *Bifidobacteria* that are residents of the commensal bacteria in the gut of humans showing good therapeutic functions (Lavanya *et al*, 2011). They can produce antimicrobial substances such as organic acid, hydrogen peroxide, and bacteriocins, which can influence the growth of possible harmful microorganisms.

Milk includes a wide variety of proteins that provide protection towards enteropathogens or are crucial for the manufacture and characteristic nature of certain milk products. Milk has been shown to contain wide varieties of bioactive compounds, which extend the range of influence of mothers over young beyond nutrition (Gobbetti *et al*. 2007). Researchers for the last decade have verified that these bioactive peptides possess very vital biological functionalities, which include antimicrobial, antihypertensive, ant oxidative, ant cytotoxic, immunomodulatory, opioid, and mineral-carrying activities.

The objective of the present study was to isolate, identify, detect the secondary metabolites secreted by *Lactiplantibacillus plantarum* and its antimicrobial, anticancer activity obtained from indigenous cow milk.

MATERIALS AND METHODS

Collection of milk sample

Raw cow milk sample was collected from the local area of Eachanari, Coimbatore-641021, India. During lactation period the milk was collected under aseptic conditions in a sterile screw cap tube and processed within few hours and used for further studies.

Isolation and conformation of *Lactobacillus sp*

Coagulated milk samples were then serially diluted and plated in to MRS agar and incubated at 37°C for 24-48h in order to obtain enriched cultures. (De Man *et al*. 1960).

Confirmation of bacteria using specific medium

The MRS agar was taken and sterilized at 121°C for 15mins. After sterilization the media was poured to petri plate and allowed to solidify, then. These cultures were quadrently streaked on MRS agar medium and incubated The plate was incubated at 37°C for 24 hours. (Hartemink et al, 1997).

Biochemical Tests

Gram staining test

The isolated bacteria were examined using gram staining kit (Becton, Dickinson and Company, USA) according to Collins and colleague's technique **(Collins, 2014),** and was observed under light microscope with a magnification of 1000x.

Bile salt tolerance

The tolerance of lactobacilli to bile salts (BS) was evaluated in MRS supplemented with bile salts using a modified method described by Dora and Glenn (2002). Test lactobacilli isolates cultures were grown for 6h in MRS broth at 37°C. culture was inoculated into 100ml MRS broth with 0.2 or 0.4% (w/v) bile salts.

NaCl tolerance

For the determination of NaCl tolerance test, MRS broth were adjusted with different concentration (1-5%) of NaCl and after sterilization, each test tube was inoculated with 1% (v/v) overnight culture of *lactobacillus* and incubated at 37°C for 24 h. After 24 h of incubation the growth was determined by observing the turbidity.

Lactose utilization

Lactose utilization was determined using acid production with the selected bacterial cultures and it was detected by observing the colour change in the medium. Sterilized fermentation medium (10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final pH 7.0) was inoculated with culture and incubated at 37^0C for 48 h**.** (Willem et al, 1994)

Molecular Identification Of Bacteria

Genomic DNA isolation

Genomic DNA isolation is a process of isolating DNA by rupturing the bacterial cell wall and degrade the protein content using phenol-chloroform precipitation method. (Cardinal et al. 1997) .

DNA from the bacterial genome was extracted as per standard phenol- chloroform method. Bacterial cultures were prepared and suspended in Luria-Bertani broth (Hi-Media, India) and incubated at 37°C for 24 hrs. The 24-hr old bacterial cells were pelleted by centrifugation and this was suspended in lysozyme and saline EDTA, after mixing incubated at 370C for 30 minutes. To this 150µl of 10% SDS was added and incubated at 65^0C for 15 minutes. Phenol, Chloroform and Iso-amyl alcohol in the ratio of 25:24:1 were added and undergone centrifugation. The aqueous phase was precipitated by adding double volume of isopropanol and washed with absolute ethanol. The DNA was suspended in 30µl of TE buffer and visualised under uv transilluminator (medox bio)

Polymerase chain reaction and sequencing

The 16SRNA study was conducted to identify the LAB as follows

PCR reaction was performed in a gradient thermal cycler (AB Applied Biosystems-Veriti 96 well Thermal cycler). The universal primers 16S Forward Primer: 5'- AGAGTTTGATCMTGGCTCAG -3' and 16S Reverse Primer: 5'- TACGGYTACCTTGTTACGACTT -3' were used for the amplification of the 16S rRNA gene fragment. PCR (20ul) was carried out by adding 4µl of Template DNA, 8µl of PCR master mix, 2µl of primer, 6µl of distilled water. The microfuge tube was kept in thermocycler and number of cycles were programmed as follows (Schneider. M 1994).

Initial denaturation a 95^0c for 30 seconds, denaturation at 95^0c for 1 minute, annealing at 55^0 c for 30 seconds, Extension at 72 ^{0}c for 15 seconds, Final extension at 72 ^{0}c for 1minute and was repeated for 25 cycles.0.6g of agarose was dissolved in 40ml of TAE buffer to that 40µl of EtBr was added, heated and

poured in gel tray and after solidification kept in tank with buffer 2µl of amplified DNA and DNA marker was loaded. 50V of power supply was applied. The PCR product was purified using Quiagen kit as per the manufacture instruction and sent to Chromous Biotech Pvt. Ltd Bangalore for sequencing. The sequence result was analysed by BLAST, and submitted to NCBI for accession number and the phylogenetic tree was constructed using MEGA7 -software.

Phylogenetic Analysis

The PCR product were sent to Chromous Biotech (Bangalore) and were sequenced using an ABI 3730XL capillary electrophoresis sequencing station (Applied Biosystem, USA). BLAST alignment search tool of NCBI gen bank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using software MEGA 7 software. (Haji gholizadeh et al., 2020).

Extraction Of Metabolites From *Lactiplantibacillus plantarum*

Preparation of production media

About 10% of inoculum was added to the production media for extracting the secondary metabolites. The production media contained (g/L) sodium chloride (2), Peptone(5), yeast extract (2), D-glucose(4), pH 7. After inoculation the flask were incubated at 37^0 C for 24 - 48 hrs.

Production and Partical purification of secondary metabolites

After incubation the medium was centrifuged at 8000 rpm for 5 minutes. The supernatant (crude extract) was collected in sterile container and subjected to partial purification. Secondary metabolites was precipitated from the crude extract by the addition of 70% salt ammonium sulfate [(NH4) 2SO4] in a cold condition (temperatures of 5 °C to 10 °C) while stirring gently to achieve 80% saturation and was then left overnight. The precipitate was separated from the filtrate by centrifugation. After centrifugation, the precipitate was dissolved in phosphate buffer solution and used for further study (Lee., 2006)

Purification of the secondary metabolites using column chromatography

The column chromatography was used for the purification of the compound. The column uses sephadex G for the purification of enzyme product. The column was first eluted with 50ml of the phosphate buffer (pH 6.8) to yield unabsorbed proteins, and subsequently with a linear gradient of 0~1 mol/L NaCl in phosphate buffer 0.02 mol/L, (pH 6.8) to desorb the adsorbed proteins (fractions I, II, III, IV). Each fraction was analysed by using spectrophotometric study at 280nm**.** (mohamed. Abdallah et al, 2019)

Characterisation of metabolites

TLC

TLC plate is a sheet of metal coated with silica, the mixture to be analyzed is spotted in the bottom of the plate. The TLC plate is then placed in a shallow pool of a solvent (ethyl acetate, chloroform, methanol, acetic acid, water in the ratio of 5:2:2:2:1) in a developing chamber and it slowly rises up the TLC plate. When the solvent has reached the top of the plate, the plate was removed and dried to identify the compounds. If the compounds are coloured, visualization is straight forward, UV lamp is used to visualize the compounds when spot not visible. Then the Rf value was calculated. Rf = Distance travelled by the solute / Distance travelled by the solvent. (Homans and Fuchs, 1970)

UV-Visible spectroscopy

Extract was examined using the UV-VIS spectrometer from 200 to 800 nm using UV – VIS Spectrophotometer (Labtronics LT 291) and the characteristic peaks were detected. (Gunasekaran. 2003).

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is an analytical method used to determine chemical bonds and biochemical compounds at the molecular level. In this study, cells were treated with different concentrations of *L. plantarum* secondary metabolites for 24 h and 48 h. Then, the culture medium was collected by centrifugation (1000 g , 2 minutes) and washed three times with sterile PBS solution. sample was analysed in infrared spectra (Shimadzu) in the range of about 4000 – 400 cm-1, to identify the functional group which is present in the bacterial extract. (Gerwert et al, 1999).

Application Of Secondary Metabolites

Antioxidant activity

DPPH (2,2-Diphenyl-1-picryl hydrazyl assay)

100 µl,200 µl of the bacterial extract (sample) was added to 0.1M of 0.5 ml of DPPH solution and 0.4 ml of 50 millimolar Tris hydrochloric acid, after shaking make up to 2 ml with distilled water. The tubes were incubated in room temperature for 30 minutes and the OD value was measured at 517 nm using spectrophotometer (LT 291 Labtronics micro processor). Ascorbic acid was used as a standard to calculate the mg/g of DPPH activity. (Poltanov et. al, 2009).

Total antioxidant

The total antioxidant activity of the sample was determined by using the phosphor molybdenum method. The assay was based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of green phosphate complex at acid pH 0.2 ml sample was combined with 2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The solution was incubated at 90° C for 90 mins. After cooling in room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer (LT 291 Labtronics microprocessor) against the blank. The Total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid . The calibration curve prepared by mixing ascorbic acid with methanol. (Trabelsi et al. 2016).

Screening for antimicrobial activity

Antibacterial activity was investigated against the food brone pathogens using well diffusion method. Mueller hinton agar was prepared (38 g of Mueller hinton agar media was dissolved in 1000 ml of distilled water and sterilized.) and 24hrs old culture of *Escherichia coli, staphylococcus aureus, Klebsiella pneumoniae* were swabbed on the agar plate by using sterile swab and wells were made with corck borer. 50µl of the sample was added along with positive disk ceftazidime (**Caz 10**) and distilled water as negative control. The plates were incubated at 37^0 c for 24 hours and after the incubation the diameter of the zone was measured.

Antifungal activity. Malt extract agar (50.0 g of malt extract agar media was dissolved in 1000 ml of distilled water and sterilized.) plates was swabbed with70 µL of *Aspergillus flavus* and *Aspergillus niger* culture and wells were filled with the samples. The Petri dishes were incubated at 30^0C for 5 days and the zone of inhibition was measured in mm. (Monisha . et al, 2017)

Anticancer activity

Cytotoxicity study was carried out using MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyletrazolium bromidefor) assay method by **(Jesteena Johney et al, 2017)**. For the anticancer activity, Hep G2 Cell Line was used with MTT assay method. The cell Line was purchased from National centre for cell Science, Pune, India. Hep G2 Cell Line was subcultured to DMEM medium and was allowed to grow in the CO2 incubator at 5% CO2, 80% humidity and at 37^0C for 72hrs. After incubation the live cells was confirmed by inverted microscope (Unicon) and the cells was transferred to microplate reader. The sample in the different concentration (10,20,30,40,50µl) and control (100µl of the Cell Line) was maintained in the plate and incubated in the CO2 incubator for 24 hrs. After incubation the cells was washed with DMSO and trypsin. 20µl of MTT dye was added and incubated for 4 to 24hrs and the OD was measured

using ELISA (Robonik-Readwell Touch Elisa Plate Analyser) reader at 570nm. Followed by the percentage of cell death was calculated using the following formula.

% of cell death = [Control OD –Sample OD /Control OD] × 100

RESULTS AND DISCUSSIONS

In the present study *Lactiplantibacillus plantarum* (OP535992.1) was isolated from indigenous cow milk for the production of metabolites and used for various applications. Previously inoculated MRS broth was taken as a source of inoculum. The 1ml of sample was added in to the broth medium and the colony morphologies of the isolates were visually observed on the surface of MRS agar solid medium; the colour varied from white to pale creamy, the shape was circular, and the size ranged from 0.5 to 4 mm in diameter. The isolated strain which is used for the further process. single bacterial colonies grown on MRS medium plates were cultivated on the same medium and pure cultures were subjected to standard procedures for the identification of *Lactobacillus* isolates.

Figure 1: Distinctive colony characteristics of the *Lactobacillus* grown on MRS agar medium for 48hrs at 37°C.

Biochemical Tests

Gram staining

The isolated bacteria was observed by light microscope. It is clear that the bacteria was gram positive, rod shaped (coccobacilli), occurring singly or in chains. The gram staining results indicated that the isolated bacteria could be identified as lactobacilli. This results conform to the findings of Gram-staining method reported by Sizemore et al.(1990).

Bile tolerance test

After exposure to acidic conditions, the lactobacilli isolate was assayed for bile salt tolerance (Table 2). The isolated *Lactobacillus* spp. was capacity to resist bile salts under exposure to 0.2% concentration after 24h at 37°

Table 1: assay of bile tolerance

Sl.no	Samples	OD value at 610nm
1	control	0.00
2	MRS broth +culture	0.68
3	MRS broth+culture+NaCl	0.39

Lactose utilization

In the present study the selected LAB isolate was grown in MRS broth medium supplemented with lactose and was observed for change in colour from red to yellow/orange which indicates the production of lactic acid. It was observed that LAB isolate was able to produce lactic acid from lactose. A similar observation was noted by Pundir et al. (2013) isolates using lactose medium.

Tolerance to NaCl

NaCl is an inhibitory substance which may inhibit the growth of certain types of bacteria. The lactic acid bacteria were sensitive to NaCl than it would not be able to show its activity in presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacteria. In the present study, the isolates were able to tolerate 2% NaCl, similar results was observed from the study of Chowdhury et al. 2012.

Molecular identification

Isolation of DNA and Polymerase chain reaction (PCR) Amplification of 16s rRNA gene

The Isolated genomic DNA of *Lactiplantibacillus plantarum* were confirmed with 1% agarose gel electrophoresis (Figure 2). The DNA was amplified by Polymerase Chain Reaction using 16SRNA primers. The Lane 1 was loaded with the PCR marker, the lane 2 were loaded with PCR product along with the 16SRNA primer respectively. The molecular weight of the amplified gene was observed, which showing **756**Kbs. Further the product was confirmed by sequencing analysis.

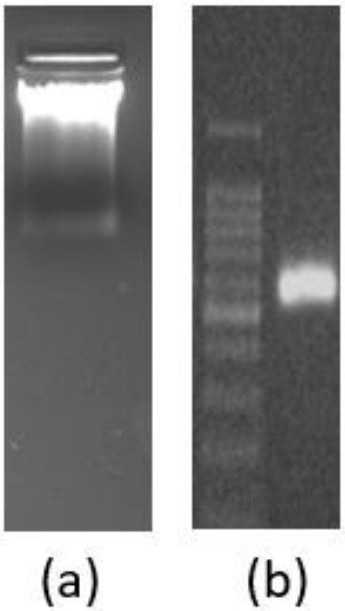

Figure 2: (a) DNA profile of *lactobacillus*
(b) DNA amplified using PCR reaction

Phylogenetic tree

A phylogenetic tree is a visual representation of the relationship between different organisms, showing the path through evolutionary time from a common ancestor to different descendants. The tips of the tree represent groups of descendent taxa (often species) and the nodes on the tree represent the common ancestors of those descendants. The gene bank accession number is **OP535992.1** (***Lactiplantibacillus plantarum***).

Saeed et al, 2020, isolated the bacteria from goat raw milk in Basrah/Iraq, which showed that the *Lactobacillus* isolates had about 99 % homology with identified *Lactiplantibacillus plantarum* recorded in the gene bank database of NCBI. The same results were obtained with the *Lactiplantibacillus plantarum* isolated by Barbosa et al 2021 from Arugula in Portugal.

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Figure 3: Phylogenetic tree based on the 16S rRNA gene sequences showing the relationships of the *Lactiplantibacillus plantarum* strain

Production and extraction of secondary metabolites

The secondary metabolites are produced by inoculating *Lactiplantibacillus plantarum* on production media. After 48 hrs of incubation the broth was centrifuged at 8000 rpm for 10 minutes at 4^0 C. The crude extract was collected in a sterile container and used for further purification. The metabolites were further partially purified using salt precipitation.

Characterisation

Chromatography

In this study, column chromatography was done with the protein extracted from ***Lactiplantibacillus plantarum***. Higher OD value obtained tube was used to pool and was used for further study (Gil-Rodriguez and Beresford 2019).

Table 2: Column chromatography

Sl.no	Samples	OD value 280nm
1	Fraction 1	0.033
2	Fraction 2	0.096
3	Fraction 3	0.085
4	Fraction 4	0.100
5	Fraction 5	0.085

The precipitated compounds were used to identify the compounds in TLC. The spots were identified on Thin Layer Chromatography by using Iodine solution (Figure 5). The corresponding Rf values of the test samples is 0.441cm

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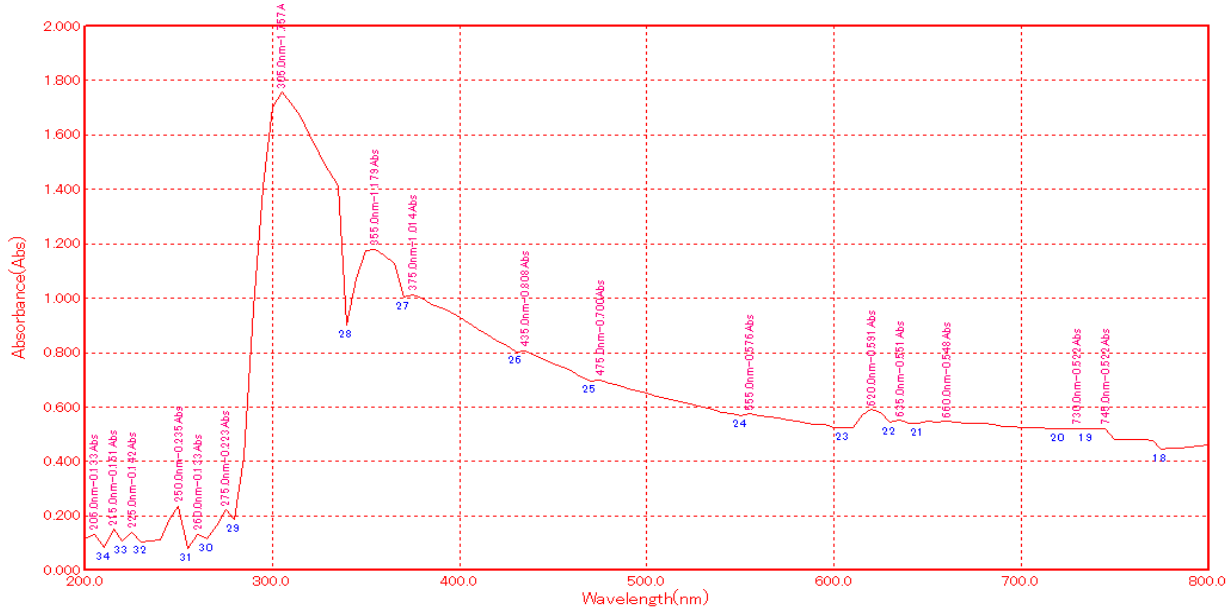
Figure 4: Thin layer chromatography

UV – Visible spectrophotometer

The characterization of surface protein extracted from ***Lactiplantibacillus plantarum*** was monitored by UV – Visible spectrophotometer analysis. The UV – Visible spectrum is obtained between 200 to 800 nm. The presence of peak at 275-305nm confirmed various bioactive compounds in the sample. Peak locations or the peak wavelengths were specific for different bioactive compound groups and the peak height depends on the concentration of the compound.

(Hamdan and Mikolajcik 1974), was purified on a Sephadex G-25 column and showed an UV spectrum with a maximum peak at 255 nm.



Graph 1: Extracted secondary metabolites peak value

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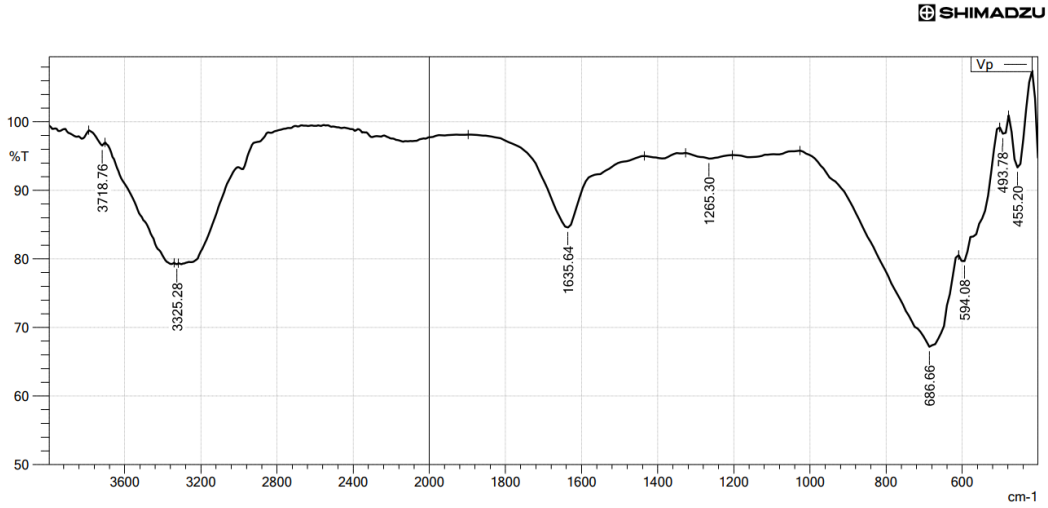
Fourier Transform Infrared (FTIR) Spectroscopy

The compound was characterized by using Fourier Transformed Infrared Spectroscopy.FTIR showed the structure, the respective bands of synthesized Metabolites.



Graph 2: FTIR Analysis

The interpretation of infrared spectra involves the correlation of absorption bands in the spectrum with that of the desired sample and the resulting band was larger macromolecular character because of the inter molecular binding of hydrogen.

FTIR result of metabolites showed the presence of carboxylic group in 3718.76cm-1 to 3325.28 cm-1 O-H stretching may be bacteriocine or niacin a, 1635.64cm-1 is an amide(c=o). 1265.30cm-1 is an carboxylic acid C-O stretch, 686.66cm-1 is an vinyl and which is also an stretching of alkene C-H which correspond to the vibrational stretching of hydroxyl group of our study. The peaks at 686.66 cm-1absorption is a strongest IR absorption and these are use full in the structure determination of metabolites and 455 cm-1 represents the C-X overlapping, the presence of alkanes.

A weak stretching band around 2943 cm-1 was assigned to the C–H stretching vibration of the aliphatic CH2 group, which indicates the presence of organic compounds like sugar, proteins, etc.(saravanan, 2016). The absorption at 1629 cm-1 may correspond to the ring stretching of galactose (Freitas, 2009).

Application Of Secondary Metabolites

Antioxidant studies

DPPH Assay

The DPPH assay was performed and the concentration of the test sample was identified using ascorbic acid as standard. The corresponding OD value was obtained using UV- Vis spectrophotometer. DPPH free radical scavenging activities indicated that the intact cells of *Lpb. plantarum* (op535992.1) showed strong antioxidant activity in vitro, which was similar to the antioxidant activity of *Lactobacillus* plantarum C88 described by (Li et al.2012)

Total antioxidant

Using phospho molybdenum method the total antioxidant activity of the sample was quantified for metabolites. Total antioxidant activity of the sample of 101mg/g.

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Table 3: The UV-Vis spectrophotometer value for the compound are as follows

Antioxidant	mg/g
DPPH Assay (100µl)	**68**
Total antioxidant	**101**

Antimicrobial Activity

Antibacterial Activity

In the antibacterial studies of *Lactiplantibacillus plantarum*, the compounds obtained from the Thin Layer Chromatography and crude samples were used (Graph 3). TLC sample showed more antibacterial activity with the three bacteria with inhibitory zones of 6 mm, 2 mm and 5mm respectively for *Escherichia coli, staphylococcus aureus, Klebsiella pneumoniae*. The crude sample shown inhibitory zones of 5mm, 2 mm and 1 mm. There was no inhibition zone for distilled water, whereas disc had the widest inhibitory zones of 7 mm, 6 mm and 4 mm respectively *Escherichia coli, staphylococcus aureus, Klebsiella pneumoniae*.

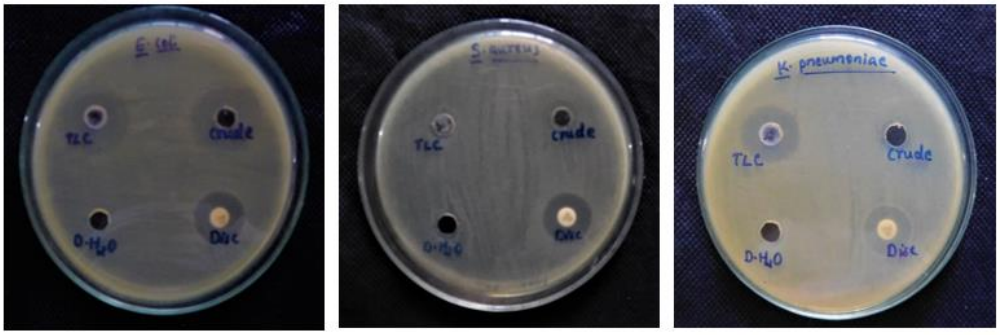
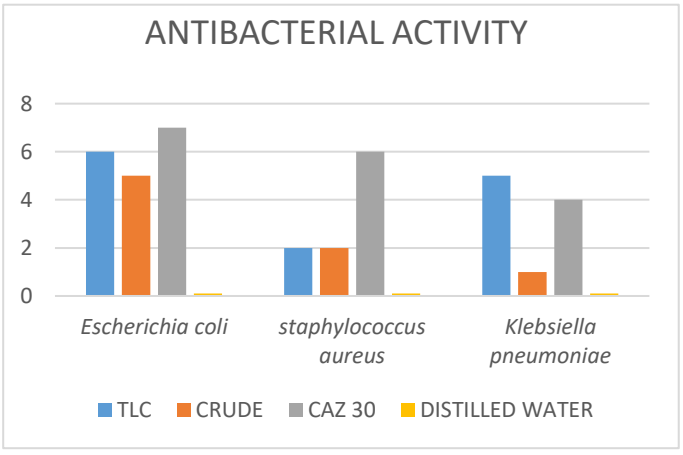


Figure 5: Antibacterial Activity by well difussion method against:*(a)Escherichia coli, (b)staphylococcus aureus, (c) Klebsiella pneumoniae*

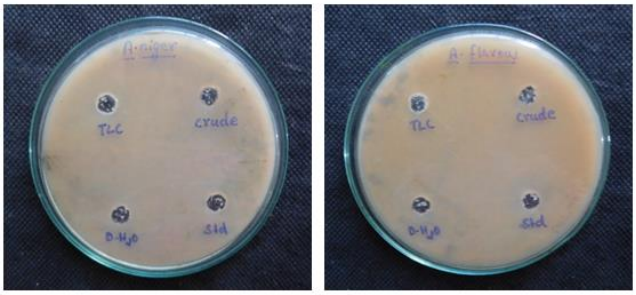


Graph 3: Antibacterial activity

Antifungal activity

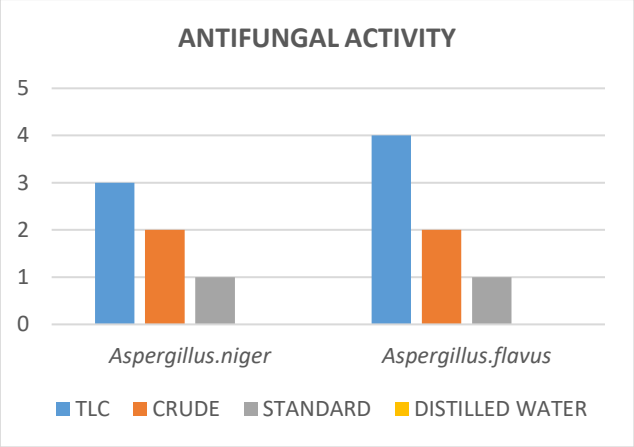
Antifungal activity of the Crude, TLC compounds was showing good result against the pathogens of *Aspergillus niger* and *Aspergillus flavus (*Grapg 4). TLC extract showed more antifungal activity with inhibitory zones of 3 mm and 4 mm. The similar results of (Lei et al., 2020;Hassan et al., 2020) found the antimicrobial activity of the extracted compound. Further more ,Sankar et al. (2012) observed that

Lactobacillus plantarum isolates from raw cow milk samples had a powerful antimicrobial activity against a set of indicator microorganisms.



(a) (b)

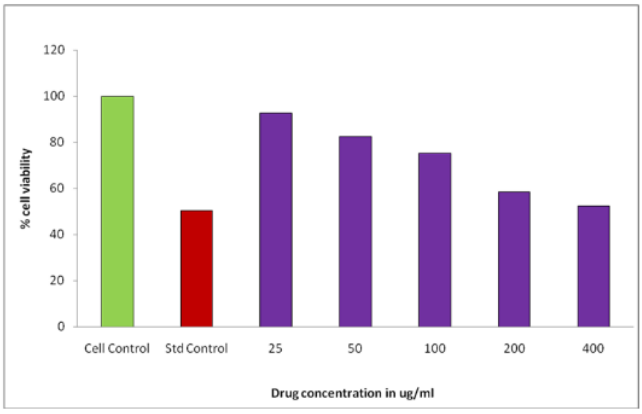
Figure 6: Antifungal Activity by well difussion method against: (a)*Aspergillus niger* and (b)*Aspergillus flavus*



Graph 4: Antifungal activity

Anticancer activity

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. Currently, *Lactiplantibacillus plantarum* bacterial compounds are being used to treat cancer effectively. Anticancer mechanisms associated with the secondary metabolite may explain the cytotoxic activity of these compounds. The selectivity of anticancer agents is significant because most of the anticancer drugs currently in use cause serious side effects.



Graph 5: Percentage of viable cell

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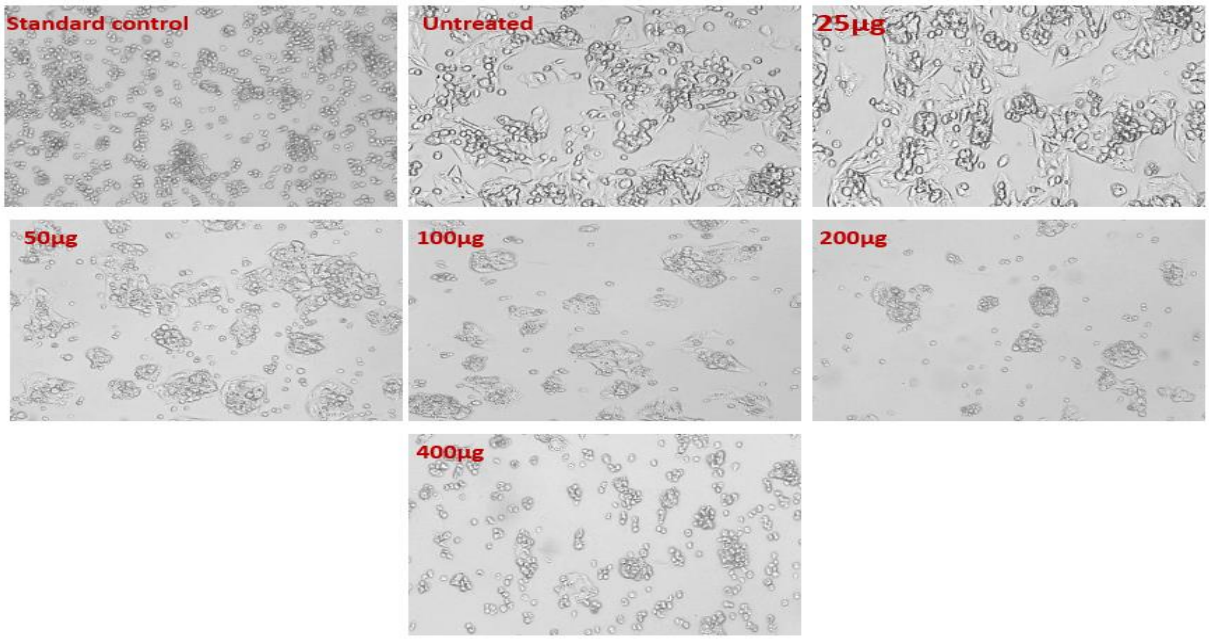

Figure 7: Results of anticancer study MTT Assay

Using MTT assay method the anticancer activity of the extracted secondary metabolites of *L. plantarum* was tested against HePG 2 cell line from various concentration (25 µg, 50 µg, 100 µg, 200 µg, 400 µg) of the sample shown (92.74%, 82.63%, 75.32%, 58.47%, 52.69%) respectively live cells observed. When compared with the control the extracted cells showed the maximum cell death (201.40 µg /ml) for 400 µg. The anticancer activity of milk peptides has been reported by several workers (Park, 2009). Several hypotheses have been proposed to explain the mechanism of the anticancer activity of milk metabolites.

CONCLUSION

In conclusion, the results obtained from this study demonstrated the potential probiotic ability of isolated LAB species from cow milk . The Genomic DNA of *lactiplantibacillus plantarum* strain was isolated and confirmed by gel electrophoresis. In PCR forward and reverse primers was used to sequence the specified gene and submitted to NCBI. The sequenced gene was identified and GenBank accession number for *lactiplantibacillus plantarum* strain (op535992.1). It has 97% similarity of 16s rRNA sequence. The metabolites have found, has antimicrobial activity, anticancerous activity, characterisation of the compounds and its various applications.

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