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MICROBIAL PRODUCTION AND NANOENCAPSULATION OF FATTY ACIDS USING *LYSINIBACILLUS FUSIFORMIS* FROM VARIABLE SOURCES OF DIETARY FIBRES: A POTENTIAL ANTIMICROBIAL AND BIOTHERAPEUTIC AGENT

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ABSTRACT

In recent times, microorganisms are utilised in the eco-friendly production of various biological compounds. One such important compound is Fatty acids (FAs). FAs are presumed to have detrimental effects on human health upon overconsumption. However, specific FAs exhibit significant antimicrobial, antioxidant and anticancer activity, thereby making them promising therapeutic agents. This study explores the microbial synthesis of FAs using the soil-dwelling bacterium *Lysinibacillus fusiformis* under anaerobic fermentation. Bacteria require dietary fibres to produce the FAs. To optimize production, cellulose, glucose, and orange peel (OP) extract were evaluated as dietary fibre substrates. Post-fermentation, the organic layer was isolated by liquid-liquid extraction. The presence of FAs was confirmed and characterized using ATR-FTIR and GC-MS. Cellulose was identified as the most efficient substrate for higher FAs yield. The FAs were stabilized by encapsulating them into Nanoparticles(NP) using sodium alginate and PVA(poly-vinyl alcohol). SEM-EDS and XRD are carried out to confirm the encapsulation of FAs into the NPs. The agar well diffusion method and MTT assay demonstrated that the FA-NPs possess potent antimicrobial and anticancer activities. Therefore, these FA-NPs represent a viable biotherapeutic agent for future

clinical applications.

KEYWORDS: Fatty acids(FAs), dietary fibres, anaerobic fermentation, FTIR-ATR, GC-MC, FAs-NP.

ABBREVIATIONS: FAs- Fatty Acids, OP-Orange Peel, ATR-FTIR -Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy, SEM-EDS-Scanning Electron Microscopy- Energy Dispersive Spectroscopy, XRD-X-ray Diffraction, FA-NPs – Fatty Acids-Nanoparticles.

1. INTRODUCTION

Microbial production is a salient biological approach for obtaining complex organic molecules from precursor molecules; the products formed are primary metabolites, which are synthesized during the exponential growth phase, and they are an integral part of the normal growth process. The resulting products can stem from both anabolic metabolism and catabolic metabolism. Industries widely target this natural machinery for the synthesis of vital commercial products, including amino acids, nucleotides, and various organic acids(Sanchez & Demain, 2008). This study specifically focuses on the microbial synthesis of Fatty Acids (FAs) by *Lysinibacillus fusiformis* using different dietary fibre sources under *in vitro* conditions. We will explore the optimal conditions required for this synthesis and the subsequent formation of nanoparticles using alginate and PVA to encapsulate and utilize the produced fatty acids. Fatty acids (FAs) are the chief organic molecules of triacylglycerols, and the second primary source of dietary energy for humans, FAs provide energy of 9kcal per gram making up to 20-35% of total calorie intake, furthermore they act as a vehicle that carry fat soluble vitamins such as A, D, E and K. They perform various important functions namely, cholesterol metabolism, constitute the cell membrane phospholipids there by providing fluidity and signalling and immune system regulation. Fatty acids can be classified into three types based on the chain length, they are SCFA(short chain fatty acids) C1-C6, MCFA (medium chain fatty acids), C7-C12; and long chain Fatty acids C13-C18 (Machate et al., 2020). The downside of FAs includes thrombosis, dysbiosis, which is an abnormal change in gut microbiota that leads to a wide range of diseases, such as gastrointestinal complications and obesity. (Maciel-Fiuza *et al*., 2023) Dysbiosis can also cause fungal infections, for instance Genus *Candida* can cause candidiasis, which affects the vagina, oral, and skin; it could also enter the bloodstream, imposing life-threatening complications.(Talapko *et al*., 2021). The human gut microbiota is the abode for diverse microorganisms, which include bacteria, fungi, viruses, and other eukaryotes that thrive to

maintain gut homeostasis. (Afzaal *et al*., 2022). The gut microbiome converts natural precursors from the host or dietary sources into a variety of lipid metabolites, such as FAs. The synthesis of FAs occurs as a result of microbial fermentation of dietary fiber (H. Zhang et al., 2024).

Dietary fibres are non-digestible carbohydrates, namely polysaccharides such as cellulose, starch, glycogen, and peptidoglycan. (Barber *et al*., 2020) For the microbial production of FAs different substrates were used, namely glucose, cellulose and orange peel. Cellulose is an unbranched, natural polymer consisting of repeated glucose units. It is the predominant polysaccharides that are present in cell walls of wood and plants, algal tissues, etc. Bacteria can also produce cellulose in the form of a nanofiber network. As a biocompatible polymer, it has broad applications in the field of biomedical (Seddiqi *et al*., 2021).

Food waste vaporization has acquired striking importance. The orange peel is considered to be the uneaten part of the citrus fruit; it is estimated that 20% of the fruit is composed of peel while the remaining is pulp, encompassing the nutrients. The orange peel contains 23% sugar, 22% cellulose, 25%pectins, and 11% hemicellulose. The orange peel can be used as a source for cellulose to produce FAs and bioethanol by following a conventional process known as fermentation. (Ayala et al., 2021a).

Bacillus spp., are aerobic or facultative anaerobic and inhabit soil, possessing a wide range of applications from enzyme production, bioremediation, and metabolite production. *Bacillus spp* is a gram-positive, rod-shaped, spore-forming bacterium that belongs to the family Bacillaceae. It represents heterogeneous groups in terms of phenotypic and genotypic characters. The inherent ability to produce a large number of secretory proteins, enzymes, and antimicrobial compounds, *Bacillus* spp., is exploited in human health-related functional food research, coupled with their enhanced tolerance and survivability under the hostile environments of the gastrointestinal tract. Besides, bacilli are more stable during the processing and storage of food and pharmaceutical preparations, making them more suitable candidates for health-promoting formulations. Further, *Bacillus* strains possess biotherapeutic agents.(Elshaghabee *et al*., 2017).

2. MATERIALS AND METHODOLOGY MATERIALS

The microbial strain was isolated from soil taken from the college campus, and the OP were obtained from the college canteen. All media used in this research study were purchased from

HI Media, and chemicals were purchased from Qualigens. Cellulose and dextrose (D-Glucose) were from SRL. Whereas Sodium alginate and PVA (polyvinyl alcohol) were brought from Sigma-Aldrich.

METHODOLOGY

2.1 Isolation of *Lysinibacillus fusiformis*

2.1.1 Sample collection and serial dilution

The soil sample was collected from the college campus in a sterile container. Serial dilution was done for the collected soil sample. It is a quantitative method that determines the microbial counts present in a particular concentration.(Ben-David & Davidson, 2014). The dilution factors are 10^{-1},10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}.

2.1.2 Pour plate technique

Nutrient agar medium was prepared by dissolving 1.68g of Nutrient agar in a total volume of 60 mL of distilled water. Post autoclave, the media was poured into sterile petri plates and allowed to solidify, then 100µl of 10^{-2}, 10^{-4}, 10^{-6} of the serial diluted samples was added onto each plate and spread out evenly with an L-rod. Incubate the Petri plates at 37°C for 24 hours to observe the colonies.

2.1.3 Streak plate technique and Gram staining

This technique is carried out to obtain a pure culture of the *Bacillus spp.*, The procedure is done by picking up the isolated colony based on morphology, which appears to be white, or creamish off-white, spherical in shape(Jamal & Ahmad, 2022) from the culture plate using an inoculating loop, and streak the culture onto the nutrient agar plate (quadrant and S-streak). Incubate the plates at 37°C and observe the results after 24 hours. Repeat this method until a pure culture is obtained. Gram staining was carried out using a staining kit from HI media to determine the presence of Gram-positive rods.

2.2 Biochemical characterization

Biochemical characterization is done to identify the species and the metabolic competence of the particular bacterial strain. The IMViC(Indole, Methyl Red, Voges-Proskauer and citric acid utilization test), catalase, oxidase, and carbohydrate fermentation test are carried out according to Bergey's manual. These tests differentiate bacteria.

2.3 Sanger- sequencing

Extraction of DNA was carried out by following the protocol of isolation of genomic DNA (Phenol-Chloroform extraction method). Gel electrophoresis was done to determine the genomic size. PCR amplification(PCR KIT from Thermoscientific) and 16S rRNA gene sequencing analysis were done. The primers used are listed below in Table 1; the amplified gene was detected by gel electrophoresis (1.5%) agarose gel. Phylogenetic analysis is carried out to determine the species of the bacterial strain.(Foysal & Lisa, 2018).

Table 1: Primers used.

Primer Details

No.	Oligo Name	Sequence (5`à 3`)	Tm (°C)	GC- Content
1	16S Forward	GGATGAGCCCGCGGCCTA	57	72.22%
2	16S Reverse	CGGTGTGTACAAGGCCCGG	58	68.42%

2.4 Microbial synthesis of fatty acids

2.4.1 Microbial synthesis of fatty acids with substrate 1% and 5% cellulose

The fermentation media components, Yeast extract(0.75g), Peptone(0.75g), Ammonium sulphate(0.45g), Dipotassium hydrogen phosphate(0.22g), Sodium chloride (0.9g), Magnesium sulphate (0.09g), Ferrous sulphate (0.045g) are weighed for 150ml of distilled water, with 1% and 5% of cellulose, equivalent to 1.5g and 7.5g, respectively. The weighed amount of cellulose is added to the labelled bottles and tightly closed. Sterilisation is carried out in an autoclave, after which the pH is adjusted to around 6-7 using diluted hydrochloric acid. Inoculate the loop of *L. fusiformis* into the bottles and mix the contents. And are placed in the anaerobic chamber under static conditions. The samples were removed after 24 and 48 hours for further processing. The composition of the fermentation broth is similar to (Atasoy et al., 2019) The above procedure is repeated by changing the substrate from cellulose to glucose-1% and OP extract to check the synthesis of FAs in different carbohydrate sources. Optimisation was carried out to determine the fatty acid produced in the fermentation broth at different times and using various substrates.

2.4.2 Microbial synthesis of fatty acid using orange(*Citrus sinensis*) peel extract as an alternative substrate

Pretreatment of orange peel

Fresh orange peels were collected from the college canteen. The orange peels were dried in the hot air oven for 3 days at a temperature of 60 °C, followed by grinding the orange peel to obtain a fine powder. Prepare 2% NaOH solution. Subsequently, weigh 10g of orange peel and transfer

it to the 2% NaOH solution, mix the contents well and then heat it at 80 °C for 2 hours in the water bath (alkali treatment). Filter the solid residue and treat it with sodium hypochlorite and heat it at 70 °C for 2 hours in the water bath. Filter the solid residue and wash thoroughly with distilled water. The above fermentation process is repeated; instead of cellulose, 1 g of orange peel extract is used.(Ayala et al., 2021b)

2.4.3 Extraction of fatty acids from the fermentation broth

Firstly, the fermentation broth samples were removed after 24 and 48 hours and then transferred to centrifuge tubes. The samples were centrifuged at 8,500 rpm for 10 minutes at 4 °C. The supernatant was removed without mixing with the pellet. The second step involves filtering the supernatant using a 0.22µm membrane filter, and acidification of the filtered sample is carried out for the stability of FAs present. The filtered supernatant was subjected to liquid-liquid extraction, carried out by the organic solvent ethyl acetate from Qualigens chemicals. The separating funnel was set up, where the sample was mixed with ethyl acetate in a ratio of 1:1. First, the aqueous sample was poured, followed by the organic ethyl acetate. The separating funnel is mixed continuously for 5 minutes, followed by a resting time for 10 minutes without any disturbance. This step is repeated twice to remove other impurities, and the extracted samples(upper organic layer) were transferred to tubes and stored in the refrigerator at 4 °C for further analysis.(Fioravante et al., 2025). A small portion of the extraction is run in a rotary evaporator to remove the ethyl acetate from the FAs, so the FTIR results would not be hindered by it.

2.4.4 Synthesis of Fatty Acid Nanoparticles

The emulsion solvent evaporation method is applied to synthesize the FA-NPs. The samples extracted using ethyl acetate are mixed well at 500 rpm for 15 minutes on a magnetic stirrer; on the other hand, 1g of alginate was dissolved in deionised water for 20 minutes at 800 rpm. Subsequently, add 1g of polyvinyl alcohol to 100 ml of deionised water and stir until PVA is completely dissolved. Slowly add the PVA to the alginate (aqueous phase) and continue stirring. Emulsion formation is carried out by slowly adding the organic phase FAs with ethyl acetate into the aqueous phase under magnetic stirring for 30 minutes. Use the sonicator to create a fine oil-in-water emulsion. Sonicate for 15 minutes. Centrifuge at 10,000 rpm for 10 minutes at 4 °C. Resuspend the pellet in deionised water and repeat the centrifugation to remove impurities. Lyophilise the sample to obtain the powder form. This procedure was modified from (Azam et al., 2022).

2.5 Analysis of Fatty acids

2.5.1 FTIR(Fourier Transform Infrared Spectroscopy)

Fourier Transformed Infrared (Shimadzu corp (00504), QATR single reflection ATR accessory was used. FTIR provides information on the functional group present in the samples, by forming peaks at a particular range, the spectra recorded between 4000 and 500 cm^{-1}, implying a bond formation. The samples, namely 1% cellulose, 5% cellulose, 1% glucose, orange peel, and avocado peel, were analysed for SCFA.(Shapaval et al., 2014)

2.5.2 Gas Chromatography- Mass spectrometry

GC-MS was carried out to determine the fatty acids present. The instrument used is Agilent Model 8890 GC System with Single Quadrupole Mass Spectrometer (5977B MSD) analyzer and the column DB-WAX(DB-17 capillary column). The GC-MS conditions were set according to (Tong et al., 2007).

2.5.3 XRD of the synthesized nanoparticles

X-ray diffraction of the synthesized nanoparticles was scanned using Powder XRD Panalytical Xpert Pro3 at SRM SCIF facilities, to determine the crystalline structure of the nanoparticles. The 2θ scans were recorded at room temperature in a continuous scan mode with a starting position at 5.0109 and an ending position of 58.3950.(Azam et al., 2022)

2.5.4 SEM(Scanning electron microscope) -EDS

The surface morphology and elemental composition of the Fatty acid nanoparticles was examined by HESE(Hi-Resolution SEM) coupled with EDS(Energy Dispersive X-ray spectroscopy detector Bruker Xflash detector 610 M). (Ismail et al., 2019)

2.6 Antimicrobial assay

2.6.1 Antibacterial assay

The agar well diffusion method was carried out for fatty FAs samples produced from cellulose, orange peel extract and glucose, using. Muller-Hinton agar and tested against *Staphylococcus spp, Enterococcus spp, Klebsiella spp, Pseudomonas spp.* 4 Wells were punctured using a gel puncher, and 100µl of cellulose, glucose, and orange peel samples were added positive control is ampicillin. The plates were incubated at 37°C for 24 hours, and the result was observed.(Thananimit et al., 2022).

2.6.2 Anti fungal assay

To check the antifungal activity, an assay was performed for the same samples using PDA agar and tested against *Aspergillus spp*. and *Candida spp*. The control used was fluconazole. The procedure is the same as that of the antibacterial assay; however, the incubation period for the antifungal assay is 48 hours.(Bhattacharyya et al., 2020)

2.7 Antioxidant Assay

Antioxidant assay was done using DPPH solution to check scavenging activity, where 3 mL of 0.1mM of DPPH was added to 2ml of the samples, and then incubated in the dark for 30 minutes at room temperature. The reduction of DPPH free radical was measured using a spectrophotometer by reading the absorbance at 517 nm (ALENCAR et al., 2018). The control is ascorbic acid. The % of total scavenging activity is calculated using the formula.

$$\% \text{ of total scavenging activity} = \frac{\text{absorbance of control- absorbance of test}}{\text{absorbance of control}} \times 100$$

A graph was plotted using the formula, the concentration of the sample on the x-axis and % of total scavenging activity on the y-axis.

2.8 Cytotoxicity assay

Cytotoxicity assay was carried out using the VERO cell line, the samples were added and incubated for 24 hours in a CO_2 incubator at 37^0C, post incubation the samples were removed from the 96-well plate and washed with phosphate-buffered saline. 100 µl of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added to the well and incubated for 4 hours. 1ml of DMSO was added to the well after incubation. Using an ELISA reader, absorbance was measured at 570nm, (Senthilraja & Kathiresan, 2015) The percentage of cell viability can be calculated using the formula.

$$\% \text{ of cell viability} = \frac{\text{Absorbance at 570nm of treated cells}}{\text{Absorbance of control cells}} \times 100$$

A graph was plotted using the formula, the concentration of the sample on the x-axis and % of cell viability on the y-axis.

2.9 Anti-cancer test

The anti-cancer activity was carried out using the breast cancer cell line MCF-7. The MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide) assay was performed to

determine cell viability. The protocol was carried out according to (Roopashree et al., 2024). A graph was plotted using the formula, with the concentration of the sample on the x-axis and %of cell viability on the y-axis.)

$$\% \text{ of cell viability} = \frac{\text{Absorbance at 570nm of treated cells}}{\text{Absorbance of control cells}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Isolation of *Lysinibacillus fusiformis*

3.1.1 sample collection and serial dilution

Soil is the abode of the diverse biological communities that possess microorganisms, pests, and plants, Unique and beneficial microorganisms can be isolated from soil and could be harnessed for our use. Serial dilution (Fig.1) is a quantitative method that determines the microbial counts that are present in a particular concentration.(Ben-David & Davidson, 2014), it also regulates the microbial load i.e., the higher the dilution factor lower the microbial load, and more isolated colonies could be obtained.

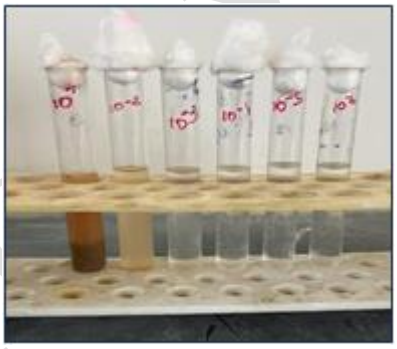


Fig.1 Serial dilution of the collected soil sample.

3.1.2 Pour plate technique

The pour plate technique was carried out for 10^{-2}, 10^{-4}, and 10^{-6} of dilution factor, mixed colonies were observed in the culture plates, and from these plates, isolated colonies were picked based on *Bacillus* morphology, which are opaque white colonies.(LU *et al*., 2018) And streaked to procure a pure culture strain.

3.1.3 streak plate technique and Gram staining

A pure culture of *Bacillus spp* was isolated by streaking on the nutrient agar plates (Fig.2). By streaking a single isolated colony, we can procure the culture plate with the specific bacterial culture, which can be primarily confirmed by Gram staining. The isolated streaked colonies

were smeared and stained to confirm the purple rod-shaped Gram-positive bacteria (Fig.3), which are *Bacillus spp*. Gram staining is based on the cell wall composition of the bacterial organism. Thick layers of peptidoglycan, which are present in Gram-positive bacteria, stain purple due to the interlocking of crystal violet and iodine complex in the cell.

Fig.2: S- streak plate of *Bacillus spp.*,

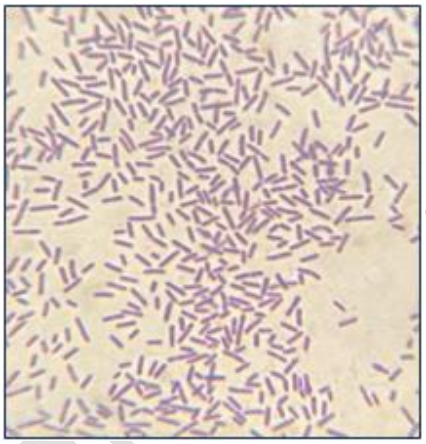


Fig. 3: Gram staining of *Bacillus spp.*

3.2 Biochemical characterisation

The *Bacillus spp* usually is positive for Indole, VP; however, this particular strain was negative for both tests. On the other hand, this strain was positive for gelatin, catalase, oxidase test and also showed positive results for citric acid utilization and carbohydrate metabolism. The biochemical characterization results are listed in Table 2 (Jinka, 2020)

Table 2: Biochemical characterization for *Bacillus.*

TEST	RESULT
Indole	Negative
Methyl red	Negative
Voges-Proskauer	Negative
Citric acid utilization	Positive
Gelatin	Positive
Catalase	Positive
Oxidase	Positive
Carbohydrate metabolism	Positive with no gas production

3.3 sanger sequencing

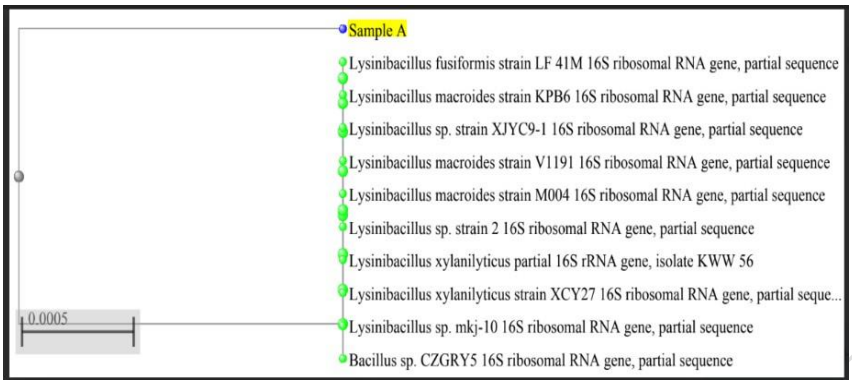


Fig. 4: phylogenetic analysis of *Lysinibacillus fusiformis.*

The Bacillus strain is identified as *Lysinibacillus fusiformis* strain LF 41M, which is a soil bacterium, rod-shaped, Gram-positive and spore-forming. In recent years, *Lysinibacillus spp* have been used in several biotechnological processes to produce metabolites, organic acids and enzymes. (Passera et al., 2021) The phylogenetic analysis of the strain is depicted in (Fig.4).

3.4 Microbial synthesis of Fatty Acids

3.4.1 Microbial synthesis of FAs with substrate 1% and 5% cellulose

Cellulose is a dietary fibre composed of polysaccharides. The building block of cellulose is D-glucopyranose, linked by β-1,4-glycosidic bonds (Revathi *et al*., 2025). *L fusiformis* is cultured in the fermentation broth of varying cellulose concentrations, such as 5% and 1%, the fermentation process was carried out for 48 hours, and samples were removed at 24hours and 48hours respectively.

3.4.2 Microbial synthesis of FAs using orange(*Citrus sinensis*) peel extract

Different sources of cellulose as substrate were used in the fermentation, namely orange-peel powder, the fresh orange peel was collected from the college canteen. The peels were dried and pre-treated and used as a source of dietary fibre for microbial synthesis of Fatty Acids. The orange peel is composed of sugar -23%, cellulose -22% , pectin -25% and finally 11%of hemicellulose.(Ayala et al., 2021b) **(**fig 6).

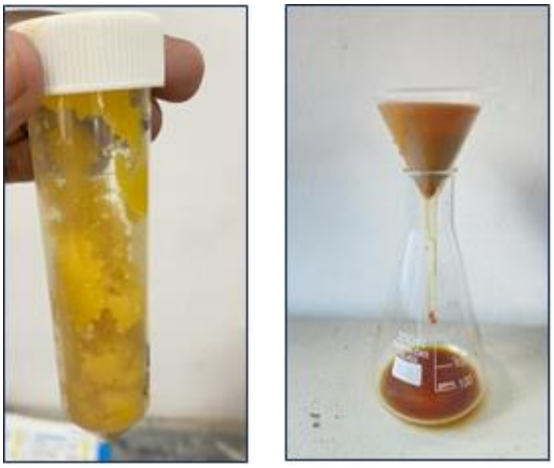


Fig. 6: Extraction of orange peel and orange peel residue.

3.4.3 Extraction of fatty acids from the fermentation broth

The collected samples were centrifuged to remove cell debris and other denser components. The supernatant was transferred to a centrifuge tube and the pellet was discarded. Liquid-liquid extraction was carried out using ethyl acetate (fig 7). Ethyl acetate, a volatile and water-immiscible solvent suitable for extracting. The extraction is carried out by acidifying the centrifuged samples (S. Zhang et al., 2019). The extracted FAs in ethyl acetate are stored at 4°C.

Fig. 7: liquid-liquid extraction of fatty acids present in the sample.

Synthesis of Fatty Acid Nanoparticles

The FA-NPs are synthesised using fatty acids obtained from different sources, namely cellulose, orange peel extract and two polymers namely alginate, a biodegradable and biocompatible biopolymer and PVA(polyvinyl alcohol), a synthetic polymer, together with alginate assist in achieving beneficial physical properties. This amalgamation of polymers is useful in drug delivery and efficiently encapsulates the FAs.(İnan & Özçimen, 2021a) (Fig 8)

Fig 8 synthesized FA-NPs.

3.5 Analysis of Fatty acids

3.5.1 FTIR analysis of Fatty acids

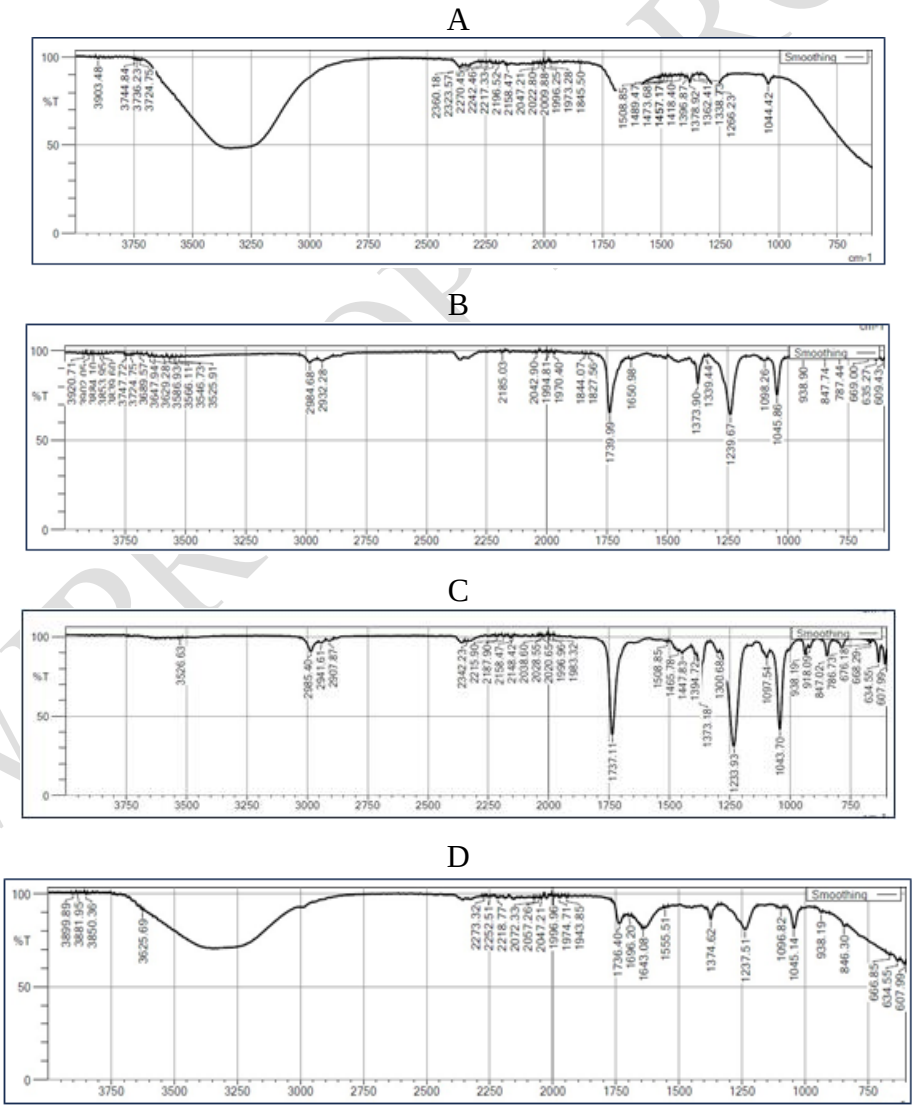


Fig. 9: FTIR analysis of Fatty Acids in different samples. A-1% Glucose, B- 1% cellulose, C- 5% cellulose, D-orange peel extract.

Table 3: FTIR data of different samples. A-1% Glucose, B- 1% cellulose, C-5% cellulose, D- orange peel extract.

A

SI.no	Wave number (cm)	Functional group
1	1338.73	C-O bond
2	1378.92	C-O bond
3	1362.41	C-O bond
4	1266.23	C-O bond

B

SI.NO	Wave number (cm)	Functional group
1	1045.86	C-O bond
2	1098.26	C-O bond
3	1739.99	C=O
4	2984.68	C-H bond
5	2932.28	C-H bond

C

SI.NO	Wave number(cm)	Functional group
1	1043.70	C-O bond
2	1097.54	C-O bond
3	1737.11	C=O bond
4	2985.40	C-H bond
5	2907.87	C-H bond

D

SI.NO	Wave number(cm)	Functional group
1	1045.14	C-O bond
2	1096.82	C-O bond
3	1736.40	C=O bond

FTIR analysis showed the presence of FAs that are produced in the various substrates used in the biosynthesis of FAs. The frequency range, wave number in which the functional groups that identify FAs are 1,700-1750 cm^{-1} C=O fatty acid group, 1000- 1200 cm^{-1} C-O, 2800-3000 cm^{-1} C-H alkane group stretching, respectively. In 1% glucose substrate sample (fig 9A) the peaks were formed at 1338.73, 1378.92, 1362.41 cm^{-1}, which indicates C-O stretching. For 1% cellulose substrate (fig 9B) the peaks of C-O bond stretching are 1045.86and 1098.26, while the C-H bond stretching is 2932.28, 2984.68 cm^{-1} and the wave number 1739.99 cm is for C=O group.

In 5% cellulose (fig 9C) substrate, the frequency range for C-O is 1043.70, 1097.54 cm^{-1}. Similarly, for the C-H bond, the ranges are 2985 and 2907 cm-1, and for the C=O bond, it is 1737.11 cm-1. Orange peel substrate (fig 9D) showed a peak at 1736.40, 1045.14, 1096.82 cm^{-1}, indicating C=O and C-H bond stretching.

The results obtained were similar for FA starch, (Abdul Hadi *et al*., 2020), in which the carbonyl stretching of the C=O bond is in the range from 1781 to 1690 cm^{-1} and in the determination of free fatty acids in Swiss cheese, where strong bands were seen in the range 1,745 cm^{-1} carbonyl groups, 1,460 C-H bending, and 1,175 cm^{-1}C-O stretching. (Koca *et al*., 2007)

The FTIR analysis shows that the presence of FAs is more in 5% cellulose, followed by 1%

cellulose and OP extract, while a minimal presence is detected in glucose. 48-hour samples showed significant peaks(Fig 9)in comparison to 24hr samples. (Rasi et al., 2022)

3.5.2 Gas Chromatography and Mass-Spectrometry

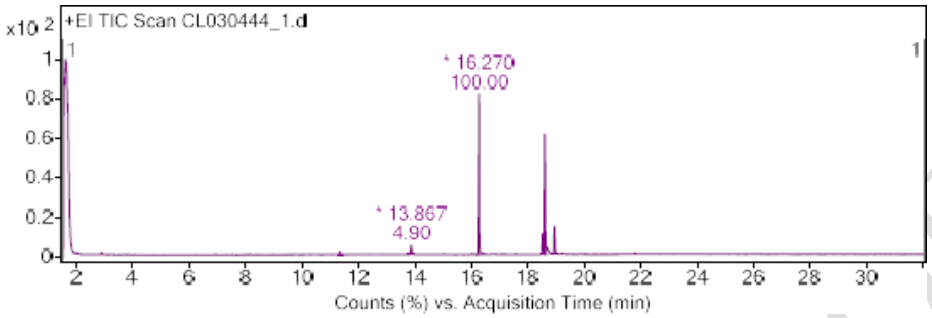


Fig 10: FA present in cellulose substrate fermentation broth.

Table 4: Retention time – FA present in cellulose substrate fermentation broth.

RT	Compound	No. of C
11.337	Lauric Acid	C12:0
13.867	Myristic Acid	C14:0
16.27	Palmitic Acid	C16:0
18.523	Linoleic Acid	C18:2
18.601	Oleic Acid	C18:1
18.939	Stearic Acid	C18:0

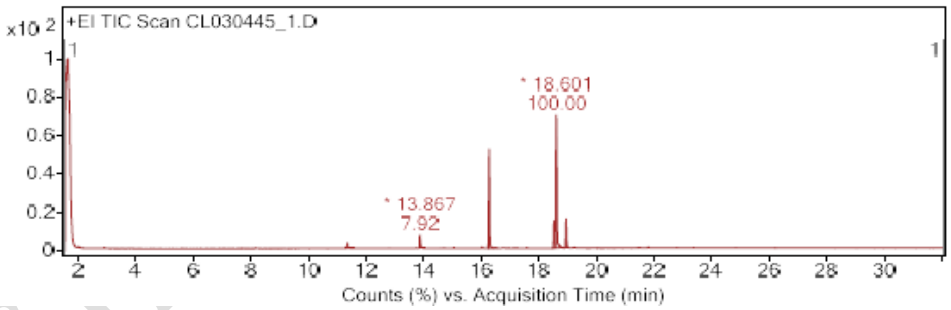


Fig 11 FA present in orange peel substrate fermentation broth.

Table 5: retention time- FA present in orange peel substrate fermentation broth.

RT	Compound	No. of C
11.337	Lauric Acid	C12:0
13.867	Myristic Acid	C14:0
16.27	Palmitic Acid	C16:0
18.529	Linoleic Acid	C18:2
18.601	Oleic Acid	C18:1
18.939	Stearic Acid	C18:0

The Fatty acids present in the cellulose substrate sample are myristic acid(C14) and Palmitic acid (C16) (Fig 10), whereas in the orange peel extract substrate, oleic acid (C18) is present (Fig 11). The retention times were compared with the NIST library, and were identified by the base peak.(Kilulya et al., 2011). Myristic acid, a saturated fatty acid, has anticancer activity that demonstrates cytotoxicity to tumour cells, and it also helps to strengthen the skin barrier. Furthermore, palmitic acid expresses anti-tumour effects by inducing apoptosis through the mitochondrial pathway by producing ROS. It prevents alveolar collapse. And finally, Linoleic acid (LA) is an omega-6 polyunsaturated fatty acid (PUFA) that improves cardiometabolic health and prevents atherosclerosis.(Fhu & Ali, 2021)

3.5.3 XRD

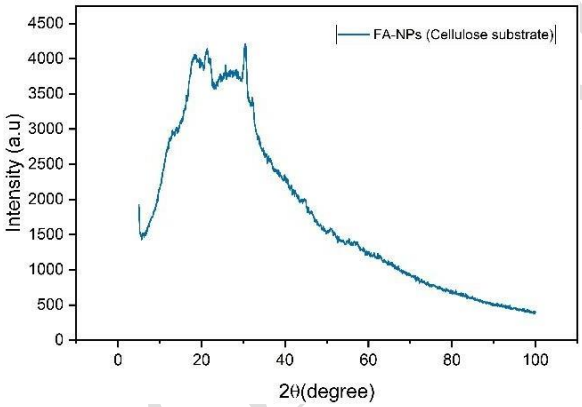


Fig. 12a: XRD pattern of FA-NPs(FA obtained from cellulose substrate, Fermentation broth).

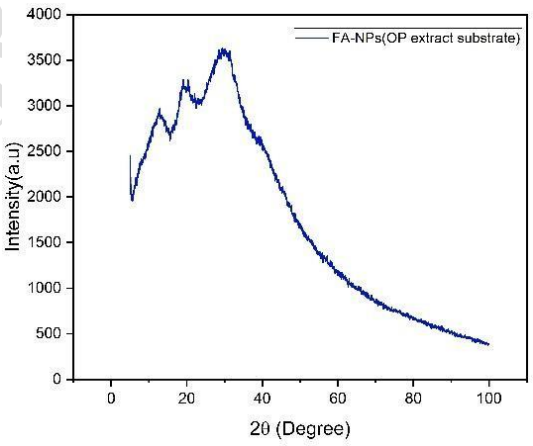


Fig. 12b: XRD pattern of FA-NPs(FA obtained from OP extract substrate Fermentation broth)

XRD patterns of the Alg-PVA-Fa were represented in Fig 12, where Fig 12a presents FAs obtained from fermentation broth with cellulose as a substrate, whereas Fig 12b depicts the

FAs from OP extract as substrate. Intense peaks were seen in Fig 12a at 2θ Values of 21.38˚, 22.12˚, 25.62˚, 28.19˚, 30.54˚, 32.22˚, 36.36˚. On the other hand, in Fig 12b the intensity peaks were relatively small, indicating its amorphous nature. Similar results were obtained in (Alva et al., 2017). The PVA and alginate influence the broad and weak diffraction peaks formed, which happens due to the strong intermolecular and intramolecular H-bonding that occurs between the polymer and chain, thereby indicating a crystalline peak. In addition, the intensity of the peak can also vary with the concentration of the polymer used.(İnan & Özçimen, 2021b)

3.5.4 SEM-EDS

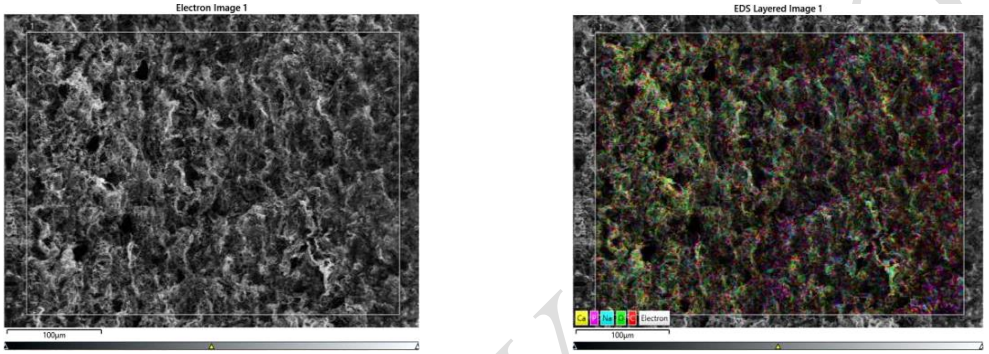


Fig 13 a SEM-EDS of FA-NPs (FA obtained from cellulose substrate Fermentation broth).

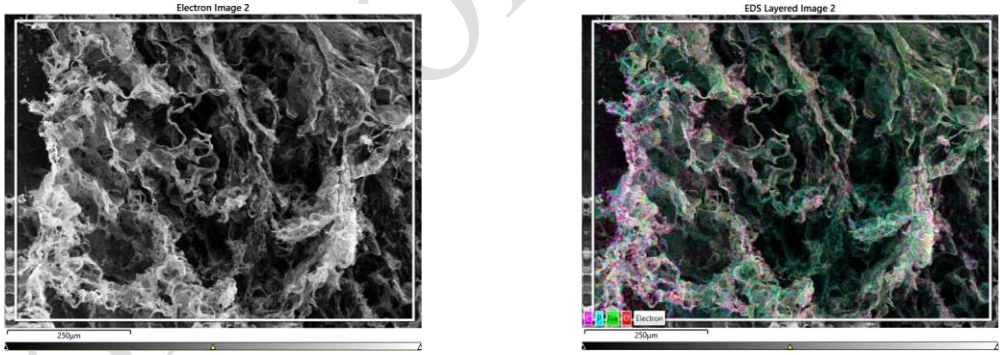


Fig 13b FA-NPs (FA obtained from OP extract substrate Fermentation broth).

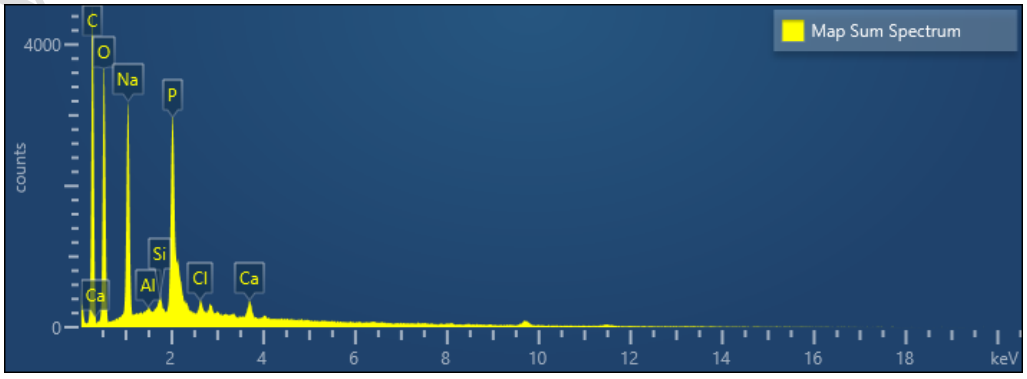


Fig. 13c: EDS spectrum.

The SEM-EDS of Alg-PVA-FA images are depicted in Fig 13a-c. Fig 13 a and b depicts the electron and EDS layered image of the surface morphology of the synthesized NP which is flake like formation. Furthermore, EDS image shows the elemental composition of the NPs majorly Carbon, Oxygen, Sodium, and Phosphorus. The presence of C and O confirms the presence of Fatty acids, whereas Na is present due to the use of Sodium alginate.

The EDS spectrum provides data on the peaks is seen in fig 13 c and Table 8 show the weight and atomic %. The EDS result shows weight% of 55.57% for C,32.18% for O element and 6.99% for Na, while certain other elements are seen in the spectrum which could be due to sample processing. While the findings are different in comparison to (Țăin (Anastasiu) et al., 2025) which used liposomes as the main component in their NP synthesis, and in this study, the fatty acids are utilized.

Table 6: the weight and atomic % of the elements present in EDS.

Element	Weight %	Atomic %
C	55.57	65.10
O	32.18	28.30
Na	6.99	4.28
Al	0.10	0.05
Si	0.25	0.12
P	3.94	1.79
Cl	0.38	0.15
Ca	0.59	0.21
Total	100.00	100.00

3.6 Anti-microbial assay

3.6.1 Anti- bacterial assay

Zone of inhibition(Fig 14) was seen in the FA-NP synthesized from cellulose substrate in *Enterococcus spp, Klebsiella pneumoniae*, and *Escherichia coli,* which is 3mm, 10mm and 3.5mm, respectively. In *Klebsiella pneumoniae* the FA biosynthesized from glucose and orange peel substrates shows a zone of inhibition of about 10mm and 6mm. (Table 7)

Similar results were observed in (Machado et al., 2021) Where FA kills or slows the growth of organisms, namely, *Enterococcus faecalis, Pseudomonas aeruginosa, and Mycobacterium tuberculosis.* The FA diffuses across the cell membrane of Gram-negative bacteria and changes the pH conditions in the cytoplasm, causing acidic stress and hindering the growth of the organism.

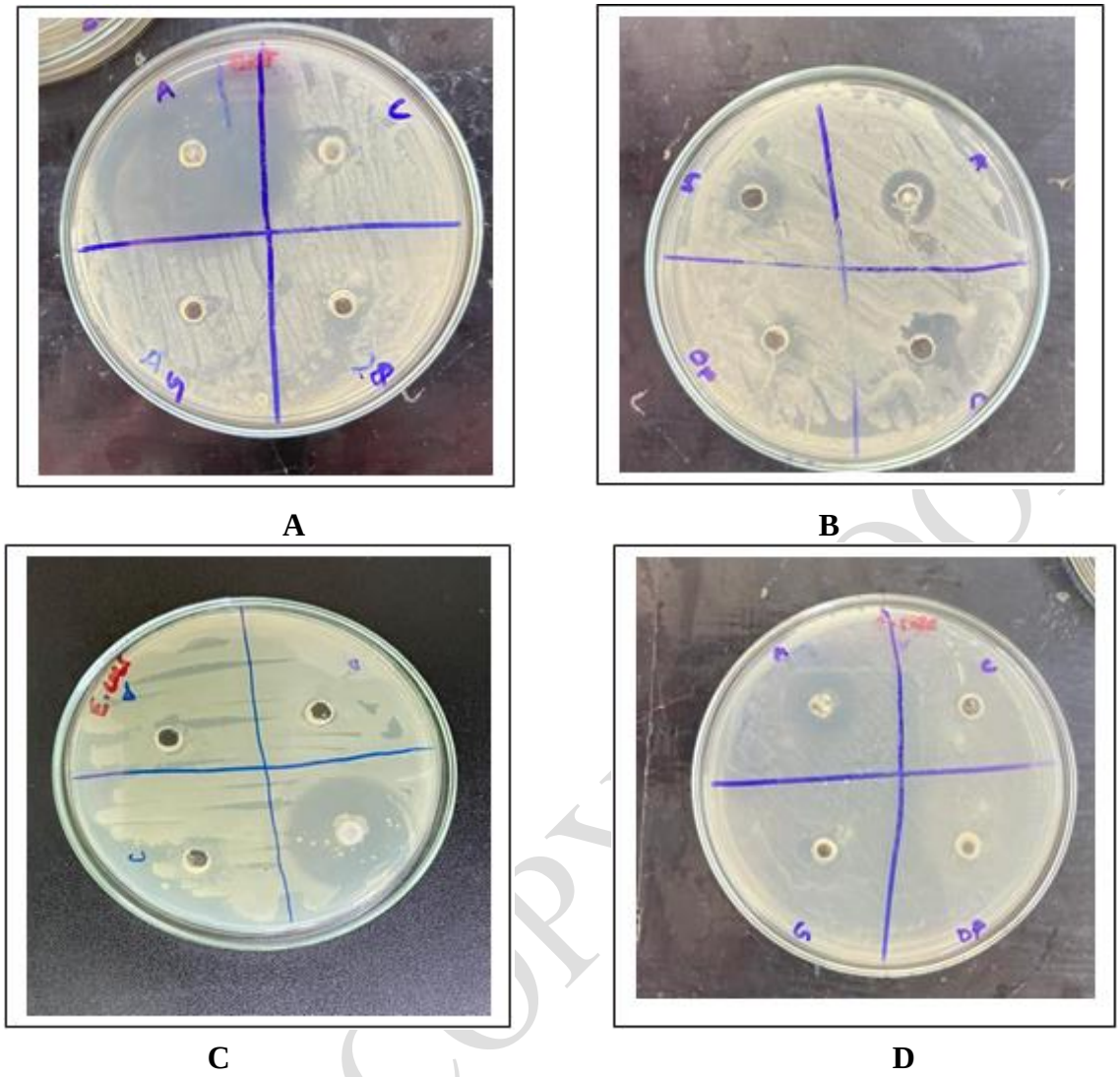


Fig. 14: Antibacterial assay for the samples cellulose glucose and orange peel extract.

A- *Enterococcus spp*, B-*Klebsiella pneumoniae*, C-*Escherichia coli*, D- *Staphylococcus aureus.*

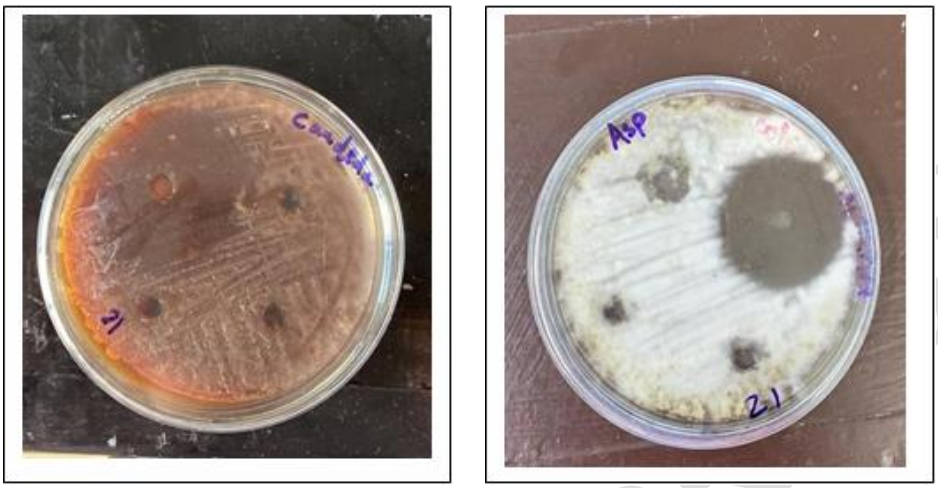
Table 7: zone of inhibition for different samples in the tested microorganism.

SI NO.	Organisms	Cellulose	glucose	Orange peel
1	*Enterococcus spp*	3mm	-	-
2	*Klebsiella pneumoniae*	10mm	10mm	6mm
3	*Escherichia coli*	3.5mm	-	-
4	*Staphylococcus aureus*	-	-	-

3.6.2 Antifungal assay

In the antifungal assay zone of inhibition(Fig 15) was seen in the FAs synthesized from cellulose as substrate in both *Candida*, where it is 16.5 mm, and *Aspergillus spp, which* is 6.5mm.(Table8) generally FAs are known to inhibit *candida* wherein the organism uses fatty acids as an alternative carbon source, which is required by processes such as gluconeogenesis

and fatty acid β-oxidation, leading to a metabolic shift from fermentative to a respiratory state, thereby affecting the cell wall structure that resists various antifungal drugs. This can potentially be therapeutic for candidiasis. (Baldewijns et al., 2021)



A **B**

Fig. 15: Anti-fungal assay the samples cellulose glucose and orange peel extract A-*Candida spp,* B-*Aspergillus spp*.

Table 8: zone of inhibition for different samples in the tested microorganism.

SI NO	Organisms	cellulose	Glucose	Orange peel
1	*Candida spp.*	16.5mm	-	-
2	*Aspergillus spp*	6.5mm	-	-

3.7 Anti-oxidant assay

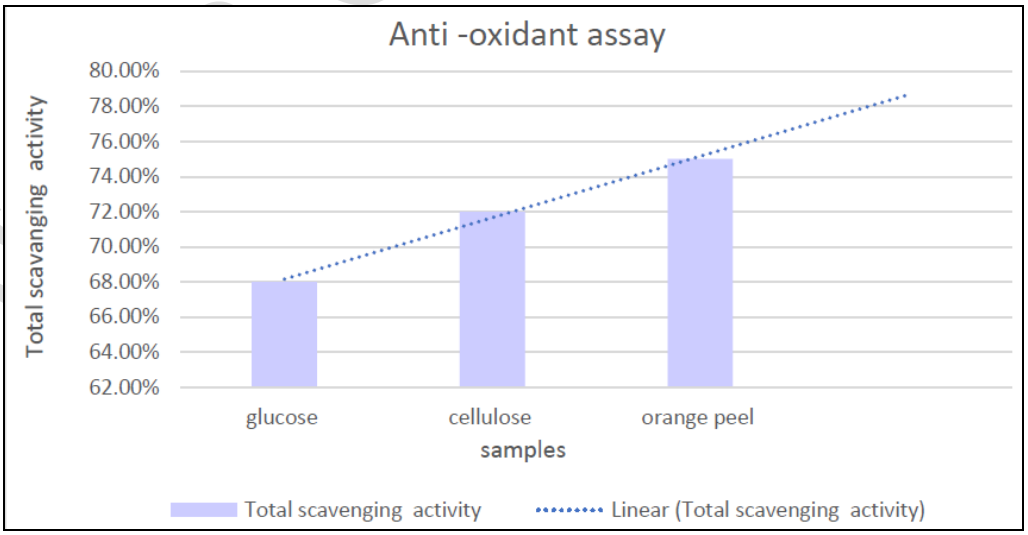


Fig 16. Total scavenging activity graph of antioxidant assay.

Table 9: Total scavenging activity of the samples.

Samples	Total scavenging activity
Glucose	68.00%
Cellulose	72.00%
Orange peel extract	75.00%

The antioxidant activity of FA was seen to be high in orange peel substrate about 75%, when compared to glucose and cellulose, which were 68% and 72%, respectively(table 11). A graph was plotted, which illustrates the data obtained. The X-axis represents the different samples, while the Total scavenging activity is on the y-axis.(Fig 16) The results obtained were similar to SCFA antioxidant activity in postbiotics (Jalali et al., 2024). The DPPH test focuses on the quantification of elements based on their reducing capacity, whether it acts with hydrogen transfer or electron transfer. Among the FAs palmitic and linoleic acid have a positive effect, wherein they reduce cholesterol(LDL) and protect ischemic stroke, as well as reduce heart diseases(Fratianni et al., 2021).

3.8 Cytotoxicity assay

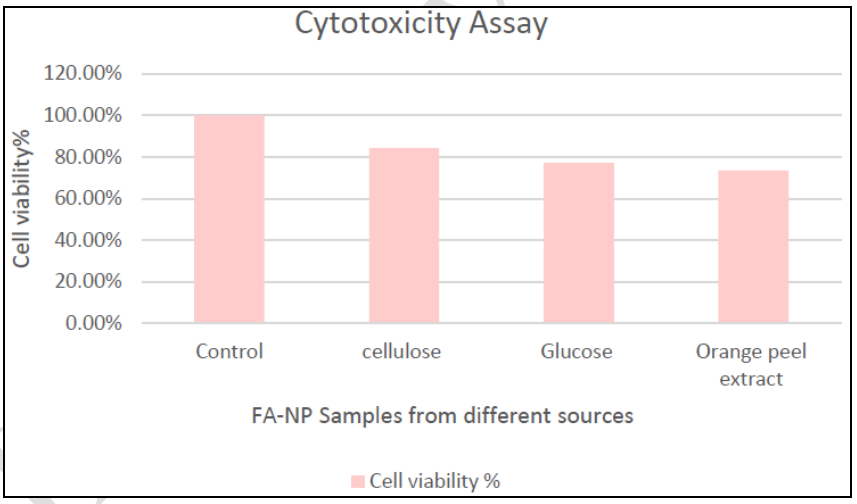


Fig. 17: Graph for the cytotoxicity assay.

Table 10: cell viability% of cytotoxicity assay.

Samples	Cell viability %
Control	100.00%
cellulose	84.23%
Glucose	77.05%
Orange peel extract	73.54%

The cytotoxicity assay was carried out for the samples and the data is represented in table 12, it clearly shows that cellulose has highest cell viability% -84.23%, while the fatty acid obtained

from glucose and orange peel extract are above 70%.). A graph was plotted, which illustrates the data obtained. The X-axis represents the different samples, while the cell viability % is on the y-axis.(Fig 17) Hence, fatty acid nanoparticles can be potent biotherapeutics in treating medical conditions and cancer. The Vero cell lines are predominantly used in cytotoxicity assays. The results differ from (Chrzanowska et al., 2022) where certain fatty acids like palmitic and linoleic acid, expressed moderate cytotoxicity and other FAs were unsatisfactory.

3.9 Anti-cancer test

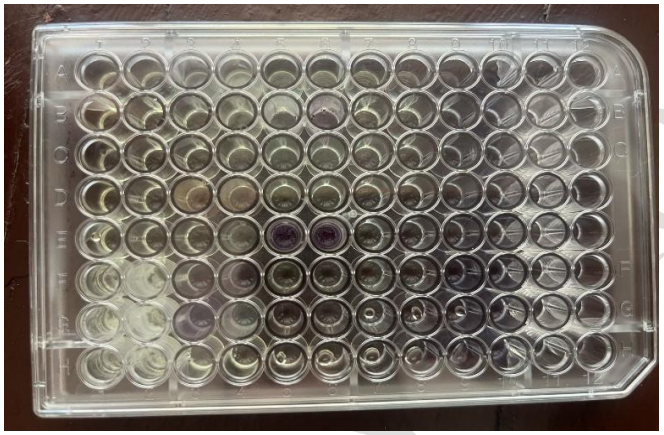


Fig. 18: 96-well plate image of MTT assay (MCF7+ breast cancer cell lines).

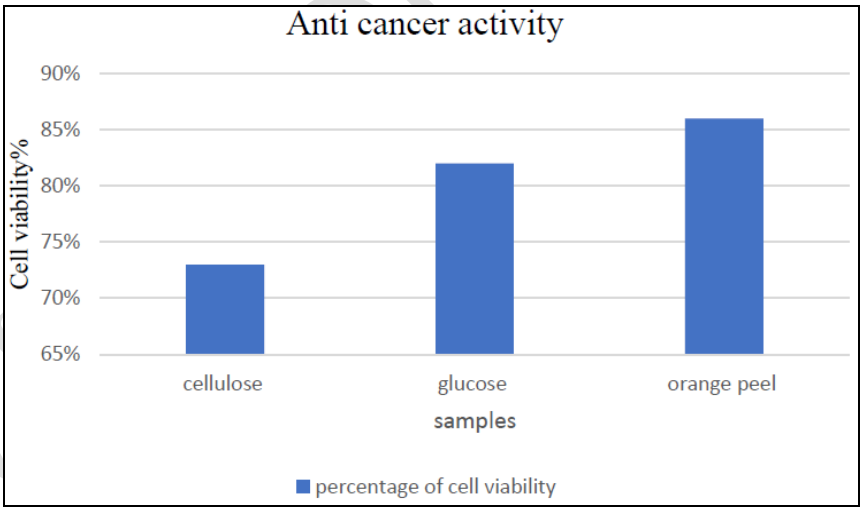


Fig. 17: Anticancer activity graph.

Table 11: Cell viability% of anticancer activity.

Samples	Percentage of cell viability
Cellulose	73%
Glucose	82%
Orange peel substrate	86%

The synthesized FA-NP from different substrates is represented on the x-axis and the cell viability% on the y-axis in the anti-cancer activity graph fig 18, and Table 13 provides the data of the cell viability% for different substrates. The FA-NP has low anti-cancer activity against MCF7+breast cancer cell lines, the percentage of cell viability was lower in cellulose substrate that is 90%, glucose and orange peel substrate was relatively low compared to cellulose. Generally Anticancer activity of FAs is more significant against colon rectal cancer lines were low cell viability% are exhibited this was mainly because the fatty acid transport system surged in several cancer cells, thereby increasing the expression of fatty acid binding proteins involved in trafficking of exogenous fatty acids, which leads to higher concentration of fatty acid in cells and result in cell death. In addition, there has been a suggestion that fatty acids such as Palmitic acid can be used as a matrix-forming agent in situ forming system for localized drug delivery for treating colorectal cancer.(Thammasut et al., 2023)

4. CONCLUSION

The exploration of *Lysinibacillus fusiformis* species as microbial factories for fatty acid biosynthesis represents a significant stride toward sustainable and potentially cost-effective production. This approach diverges from traditional chemical synthesis, offering a bio-based alternative that aligns with the growing demand for environmentally friendly processes. Throughout this investigation, it has become evident that *Lysinibacillus fusiformis* strain possesses remarkable versatility and metabolic capabilities that can be harnessed for the production of valuable FAs.

The FAs produced through *Lysinibacillus fusiformis* fermentation and synthesized into nanoparticles using alginate and PVA demonstrate promising antimicrobial and anticancer activities, highlighting their potential as therapeutic agents. Their ability to inhibit pathogen growth and induce apoptosis in cancer cells underscores their multifaceted biological functions. The FA-ALG-PVA nanoparticles synthesized from various substrates, such as cellulose and orange peel extract, showed the incorporation of FAs; however, the FAs obtained from glucose substrate showed poor incorporation and more residual contamination. Prospects lie in optimizing *Lysinibacillus fusiformis* fermentation for enhanced FA production, exploring synergistic effects with other therapeutic modalities, conducting comprehensive clinical trials to validate their efficacy and safety and synthesizing them into potential Nanoparticles which are completely devoid of elemental contamination. Further research should focus on the specific mechanisms of action, targeted delivery systems, and the development of novel FAs

derivatives to maximise their therapeutic potential in combating infectious diseases and cancer.

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REFERENCES

1. Abdul Hadi, N., Wiege, B., Stabenau, S., Marefati, A., & Rayner, M. (2020). Comparison of Three Methods to Determine the Degree of Substitution of Quinoa and Rice Starch Acetates, Propionates, and Butyrates: Direct Stoichiometry, FTIR, and 1H-NMR. *Foods*, *9*(1): 83. https://doi.org/10.3390/foods9010083.
2. Afzaal, M., Saeed, F., Shah, Y. A., Hussain, M., Rabail, R., Socol, C. T., Hassoun, A., Pateiro, M., Lorenzo, J. M., Rusu, A. V., & Aadil, R. M. (2022). Human gut microbiota in health and disease: Unveiling the relationship. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.999001.
3. ALENCAR, D., DINIZ, J., Rocha, S., Pires-Cavalcante, K., LIMA, R., SOUSA, K., FREITAS, J., BEZERRA, R., BARACHO, B., Sampaio, A., Viana, F., & SAKER-SAMPAIO, S. (2018). Fatty acid composition from the marine red algae Pterocladiella capillacea (S. G. Gmelin) Santelices & Hommersand 1997 and Osmundaria obtusiloba (C. Agardh) R. E. Norris 1991 and its antioxidant activity. *Anais Da Academia Brasileira de Ciências*, *90*. https://doi.org/10.1590/0001- 3765201820160315.
4. Alva, G., Huang, X., Liu, L., & Fang, G. (2017). Synthesis and characterization of microencapsulated myristic acid–palmitic acid eutectic mixture as phase change material for thermal energy storage. *Applied Energy*, *203*: 677–685. https://doi.org/10.1016/j.apenergy.2017.06.082
5. Atasoy, M., Eyice, O., Schnürer, A., & Cetecioglu, Z. (2019). Volatile fatty acids production via mixed culture fermentation: Revealing the link between pH, inoculum type and bacterial composition. *Bioresource Technology*, *292*: 121889.

https://doi.org/10.1016/j.biortech.2019.121889.

6. Ayala, J. R., Montero, G., Coronado, M. A., García, C., Curiel-Alvarez, M. A., León, J. A., Sagaste, C. A., & Montes, D. G. (2021a). Characterization of Orange Peel Waste and Valorization to Obtain Reducing Sugars. *Molecules*, *26*(5): 1348. https://doi.org/10.3390/molecules26051348.
7. Ayala, J. R., Montero, G., Coronado, M. A., García, C., Curiel-Alvarez, M. A., León, J. A., Sagaste, C. A., & Montes, D. G. (2021b). Characterization of Orange Peel Waste and Valorization to Obtain Reducing Sugars. *Molecules*, *26*(5): 1348. https://doi.org/10.3390/molecules26051348.
8. Azam, F., Ahmad, F., Ahmad, S., Zafar, M. S., Ulker, Z., Azam, F., Ahmad, F., Ahmad, S., Zafar, M. S., & Ulker, Z. (2022). Preparation and Characterization of Alginate Hydrogel Fibers Reinforced by Cotton for Biomedical Applications. *Polymers*: *14*(21). https://doi.org/10.3390/polym14214707.
9. Baldewijns, S., Sillen, M., Palmans, I., Vandecruys, P., Van Dijck, P., & Demuyser, L. (2021). The Role of Fatty Acid Metabolites in Vaginal Health and Disease: Application to Candidiasis. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.705779.
10. Barber, T. M., Kabisch, S., Pfeiffer, A. F. H., & Weickert, M. O. (2020). The Health Benefits of Dietary Fibre. *Nutrients*, *12*(10): Article 10. https://doi.org/10.3390/nu12103209.
11. Ben-David, A., & Davidson, C. E. (2014). Estimation method for serial dilution experiments. *Journal of Microbiological Methods*, *107*: 214–221. https://doi.org/10.1016/j.mimet.2014.08.023.
12. Bhattacharyya, A., Sinha, M., Singh, H., Patel, R. S., Ghosh, S., Sardana, K., Ghosh, S., & Sengupta, S. (2020). Mechanistic Insight Into the Antifungal Effects of a Fatty Acid Derivative Against Drug-Resistant Fungal Infections. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.02116.
13. Chrzanowska, A., Olejarz, W., Kubiak-Tomaszewska, G., Ciechanowicz, A. K., Struga, M., Chrzanowska, A., Olejarz, W., Kubiak-Tomaszewska, G., Ciechanowicz, A. K., & Struga, M. (2022). The Effect of Fatty Acids on Ciprofloxacin Cytotoxic Activity in Prostate Cancer Cell Lines—Does Lipid Component Enhance Anticancer Ciprofloxacin Potential? *Cancers*, *14*(2). https://doi.org/10.3390/cancers14020409.
14. Elshaghabee, F. M. F., Rokana, N., Gulhane, R. D., Sharma, C., & Panwar, H. (2017). Bacillus As Potential Probiotics: Status, Concerns, and Future Perspectives. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.01490.

15. Fhu, C. W., & Ali, A. (2021). Protein Lipidation by Palmitoylation and Myristoylation in Cancer. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.673647.
16. Fioravante, I. F., Nobrega, R. D. Q., & Silva, J. F. L. (2025). Recovery of Volatile Fatty Acids from Fermentation Broth Using Ethyl Acetate as Solvent. *Chemical Engineering Transactions*, *117:* 115–120. https://doi.org/10.3303/CET25117020
17. Foysal, M. J., & Lisa, A. K. (2018). Isolation and characterization of *Bacillus* sp. Strain BC01 from soil displaying potent antagonistic activity against plant and fish pathogenic fungi and bacteria. *Journal of Genetic Engineering and Biotechnology*, *16*(2): 387–392. https://doi.org/10.1016/j.jgeb.2018.01.005.
18. Fratianni, F., d'Acierno, A., Ombra, M. N., Amato, G., De Feo, V., Ayala-Zavala, J. F., Coppola, R., & Nazzaro, F. (2021). Fatty Acid Composition, Antioxidant, and in vitro Anti-inflammatory Activity of Five Cold-Pressed Prunus Seed Oils, and Their Anti-biofilm Effect Against Pathogenic Bacteria. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.775751.
19. İnan, B., & Özçimen, D. (2021a). Preparation and characterization of microalgal oil loaded alginate/poly (vinyl alcohol) electrosprayed nanoparticles. *Food and Bioproducts Processing*, *129*: 105–114. https://doi.org/10.1016/j.fbp.2021.07.008.
20. İnan, B., & Özçimen, D. (2021b). Preparation and characterization of microalgal oil loaded alginate/poly (vinyl alcohol) electrosprayed nanoparticles. *Food and Bioproducts Processing*, *129*: 105–114. https://doi.org/10.1016/j.fbp.2021.07.008
21. Ismail, R., Sovány, T., Gácsi, A., Ambrus, R., Katona, G., Imre, N., & Csóka, I. (2019). Synthesis and Statistical Optimization of Poly (Lactic-Co-Glycolic Acid) Nanoparticles Encapsulating GLP1 Analog Designed for Oral Delivery. *Pharmaceutical Research*, *36*(7): 99. https://doi.org/10.1007/s11095-019-2620-9
22. Jalali, S., Mojgani, N., Haghighat, S., Sanjabi, M. R., & Sarem-Nezhad, S. (2024). M Investigation of antimicrobial and antioxidant properties of postbiotics produced by *lactobacillus rhamnosus* and *limosilactobacillus reuteri* and their potential application in surface decontamination of red meat. *LWT*, *209*: 116758. https://doi.org/10.1016/j.lwt.2024.116758.
23. Jamal, Q. M. S., & Ahmad, V. (2022). Lysinibacilli: A Biological Factories Intended for Bio-Insecticidal, Bio-Control, and Bioremediation Activities. *Journal of Fungi*, *8*(12): 1288. https://doi.org/10.3390/jof8121288.
24. Jinka, J. G. R. (2020). *Morphological And Biological Characterisation Of Lysinibacillus*

Fusiformis L52 Isolated From Dairy Industry Wastes. *9*(01).

25. Kilulya, K. F., Msagati, T. A. M., Mamba, B. B., Ngila, J. C., & Bush, T. (2011). Imidazolium ionic liquids as dissolving solvents for chemical-grade cellulose in the determination of fatty acids using gas chromatography-mass spectrometry. *BioResources*, *6*(3): 3272–3288. https://doi.org/10.15376/biores.6.3.3272-3288.
26. Koca, N., Rodriguez-Saona, L. E., Harper, W. J., & Alvarez, V. B. (2007). Application of Fourier Transform Infrared Spectroscopy for Monitoring Short-Chain Free Fatty Acids in Swiss Cheese. *Journal of Dairy Science*, *90*(8): 3596–3603. https://doi.org/10.3168/jds.2007-0063.
27. LU, Z., GUO, W., & LIU, C. (2018). Isolation, identification and characterization of novel Bacillus subtilis. *The Journal of Veterinary Medical Science*, *80*(3): 427–433. https://doi.org/10.1292/jvms.16-0572.
28. Machado, M. G., Sencio, V., & Trottein, F. (2021). Short-Chain Fatty Acids as a Potential Treatment for Infections: A Closer Look at the Lungs. *Infection and Immunity*, *89*(9): https://doi.org/10.1128/iai.00188-21.
29. Machate, D. J., Figueiredo, P. S., Marcelino, G., Guimarães, R. de C. A., Hiane, P. A., Bogo, D., Pinheiro, V. A. Z., Oliveira, L. C. S. de, Pott, A., Machate, D. J., Figueiredo, P. S., Marcelino, G., Guimarães, R. de C. A., Hiane, P. A., Bogo, D., Pinheiro, V. A. Z., Oliveira, L. C. S. de, & Pott, A. (2020). Fatty Acid Diets: Regulation of Gut Microbiota Composition and Obesity and Its Related Metabolic Dysbiosis. *International Journal of Molecular Sciences*, *21*(11). https://doi.org/10.3390/ijms21114093.
30. Maciel-Fiuza, M. F., Muller, G. C., Campos, D. M. S., do Socorro Silva Costa, P., Peruzzo, J., Bonamigo, R. R., Veit, T., & Vianna, F. S. L. (2023). Role of gut microbiota in infectious and inflammatory diseases. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1098386.
31. Passera, A., Rossato, M., Oliver, J. S., Battelli, G., Shahzad, G.-I.-R., Cosentino, E., Sage, J. M., Toffolatti, S. L., Lopatriello, G., Davis, J. R., Kaiser, M. D., Delledonne, M., & Casati, P. (2021). Characterization of *Lysinibacillus fusiformis* strain S4C11: *In vitro*, *in planta*, and *in silico* analyses reveal a plant-beneficial microbe. *Microbiological Research*, *244*: 126665. https://doi.org/10.1016/j.micres.2020.126665.
32. Rasi, S., Vainio, M., Blasco, L., Kahala, M., Leskinen, H., & Tampio, E. (2022). Changes in volatile fatty acid production and microbiome during fermentation of food waste from hospitality sector. *Journal of Environmental Management*, *308*: 114640. https://doi.org/10.1016/j.jenvman.2022.114640.

33. Revathi, V., Bora, S., Afzia, N., & Ghosh, T. (2025). Orange peel composition, biopolymer extraction, and applications in paper and packaging sector: A review. *Sustainable Chemistry and Pharmacy*, *43*: 101908. https://doi.org/10.1016/j.scp.2025.101908.
34. Roopashree, P. G., Shetty, S. S., Shetty, V. V., Suhasini, P. C., & Suchetha, K. N. (2024). Inhibitory effects of medium-chain fatty acids on the proliferation of human breast cancer cells via suppression of Akt/mTOR pathway and modulating the Bcl-2 family protein. *Journal of Cellular Biochemistry*, *125*(6): e30571. https://doi.org/10.1002/jcb.30571.
35. Sanchez, S., & Demain, A. L. (2008). Metabolic regulation and overproduction of primary metabolites. *Microbial Biotechnology*, *1*(4): 283–319. https://doi.org/10.1111/j.1751-7915.2007.00015.x.
36. Seddiqi, H., Oliaei, E., Honarkar, H., Jin, J., Geonzon, L. C., Bacabac, R. G., & Klein-Nulend, J. (2021). Cellulose and its derivatives: Towards biomedical applications. *Cellulose*, *28*(4): 1893–1931. https://doi.org/10.1007/s10570-020-03674-w.
37. Senthilraja, P., & Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell lines study of Marine Yeast. *Journal of Applied Pharmaceutical Science*, 080–084. https://doi.org/10.7324/JAPS.2015.50313.
38. Shapaval, V., Afseth, N. K., Vogt, G., & Kohler, A. (2014). Fourier transform infrared spectroscopy for the prediction of fatty acid profiles in Mucor fungi grown in media with different carbon sources. *Microbial Cell Factories*, *13*(1): 86. https://doi.org/10.1186/1475-2859-13-86.
39. Țăin (Anastasiu), A.-E., Bîrcă, A. C., Naulea, A. M. I., Niculescu, A.-G., Grumezescu, M., Croitoru, G.-A., Țăin (Anastasiu), A.-E., Bîrcă, A. C., Naulea, A. M. I., Niculescu, A.-G., Grumezescu, A. M., & Croitoru, G.-A. (2025). Alginate/PVA Hydrogel Incorporating HA-Liposomes and Aronia-Derived Silver Nanoparticles for Advanced Wound Management. *International Journal of Molecular Sciences*, *26*(18). https://doi.org/10.3390/ijms26189203.
40. Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., & Škrlec,(2021). Candida albicans—The Virulence Factors and Clinical Manifestations of Infection. *Journal of Fungi*, *7*(2): Article 2. https://doi.org/10.3390/jof7020079.
41. Thammasut, W., Intaraphairot, T., Chantadee, T., Senarat, S., Patomchaiviwat, V., Chuenbarn, T., & Phaechamud, T. (2023). Antimicrobial and antitumoral activities of saturated fatty acid solutions. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2023.03.769.

42. Thananimit, S., Pahumunto, N., Teanpaisan, R., Thananimit, S., Pahumunto, N., & Teanpaisan, R. (2022). Characterization of Short Chain Fatty Acids Produced by Selected Potential Probiotic Lactobacillus Strains. *Biomolecules*, *12*(12). https://doi.org/10.3390/biom12121829.
43. Tong, L., Zhang, L., Yu, S., Chen, X., & Bi, K. (2007). Analysis of the fatty acids from Periploca sepium by GC-MS and GC-FID. *FID / Asian Journal of Traditional Medicines*, *2*.
44. Zhang, H., Xie, Y., Cao, F., & Song, X. (2024). Gut microbiota-derived fatty acid and sterol metabolites: Biotransformation and immunomodulatory functions. *Gut Microbes*, *16*(1): 2382336. https://doi.org/10.1080/19490976.2024.2382336.
45. Zhang, S., Wang, H., & Zhu, M.-J. (2019). A sensitive GC/MS detection method for analyzing microbial metabolites short chain fatty acids in fecal and serum samples. *Talanta*, *196*: 249–254. https://doi.org/10.1016/j.talanta.2018.12.049.