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Contact:
Gazi Üniversitesi Sağlık Bilimleri Enstitüsü
Tunus Cad. No:35 Kat:3-4 Kavaklıdere Çankaya /ANKARA
Phone: +90312 202 33 87 +90312 202 33 90 Fax: +90312 202 82 20
e-mail : guhes@gazi.edu.tr Web Sites: https://guhes.gazi.edu.tr https://dergipark.org.tr/tr/pub/guhes

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Antimicrobial Activity and Statistical Correlation Analysis of *Lactobacillus* spp. Isolated from Fermented Cassava and Corn against Pathogenic Bacterial Isolates

Olodu Blessing Adoh[1*], Enabulele Stephen Amadin[1]

[1]Department of Biological Science (Microbiology), Benson Idahosa University, Benin City, Edo State, Nigeria

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Abstract

This study focused on the antimicrobial activity and statistical correlation analysis of *lactobacillus* spp. isolated from fermented cassava and corn against pathogenic bacterial isolates.

Five (5) *Lactobacillus* strains, including *Lactobacillus fermentum*, *Lactobacillus ghanensis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and two *Bacillus* strains; *Bacillus cereus*, and *Bacillus pacificus*, others included *Lactococcus lactis* and *Lysinibacillus sphaericus*, were evaluated for inhibitory activity against a range of pathogenic bacterial isolates. These pathogens included *Candida* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Escherichia coli*, *Salmonella* sp., and *Streptococcus* sp., with inhibition zones recorded to assess the effectiveness of each strain. Among the tested strains, *Lactobacillus plantarum* demonstrated the highest antimicrobial activity, showing inhibition zones of 18.1 mm against *Pseudomonas* sp., 15.3 mm against *Candida* sp., and 14.6 mm against *Staphylococcus* sp., while *Bacillus cereus* exhibited the lowest inhibitory effects. Statistical analysis, using t-tests and paired sample correlations, revealed significant relationships between certain pathogenic pairs, such as *Candida* sp. and *Klebsiella* sp. ($r = 0.905$, $p = 0.001$) and *Staphylococcus* sp. and *Streptococcus* sp. ($r = 0.765$, $p = 0.007$), indicating shared susceptibilities. A Euclidean distance proximity matrix highlighted clustering patterns among strains, with a close distance of 1.75mm observed between *Lactobacillus fermentum* and *Lactobacillus plantarum*, suggesting similar resistance profiles, while *Lactobacillus delbrueckii* and *Bacillus pacificus* displayed a larger distance of 4.32mm, reflecting distinct profiles. In conclusion, *Lactobacillus* species from fermented foods demonstrate promising antimicrobial properties, supporting their potential role in developing probiotic-based approaches for combating pathogenic bacteria.

Olodu Blessing Adoh **(Corresponding author);** ORCID: 0000-0001-7561-3117, e-mail: blessingolodu18@gmail.com
Enabulele Stephen Amadin; ORCID: 0000-0002-0988-5664, e-mail: senabulele@biu.edu.ng

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1. Introduction

Fermented foods have long been recognized for their health benefits and are an integral part of many diets worldwide, providing valuable nutrients and beneficial microorganisms, particularly lactic acid bacteria (LAB) (Aderolake et al., 2023). Among LAB, Lactobacillus species have gained increasing attention for their probiotic properties, which include promoting gut health, modulating immune responses, and inhibiting pathogens through competitive exclusion and antimicrobial compound production (Adesulu-Dahunsi et al., 2022; Drissi et al., 2014). The exploration of Lactobacillus strains from traditional fermented foods, such as cassava and corn, is of particular interest in Nigeria, where these products play a crucial role in the daily diet and serve as sources of indigenous LAB with unique health-promoting properties (Olodu et al., 2024; Drissi et al., 2015; Olaniran et al., 2023).

The investigation into the probiotic potential of Lactobacillus species isolated from fermented foods typically involves assessing their ability to survive gastrointestinal conditions, adhere to intestinal mucosa, and produce antimicrobial agents, such as organic acids and bacteriocins (Akter et al., 2023; Assohoun et al., 2023). These characteristics not only inhibit pathogenic microorganisms but also contribute to the balance of the gut microbiome, an essential component of overall health (Dahiya et al., 2023; Dahiya et al., 2022). However, alongside these health benefits, it is crucial to evaluate the antimicrobial resistance profiles of these strains, as horizontal gene transfer of resistance traits from probiotics to pathogens remains a public health concern (Joghataei et al., 2019; Kaur et al., 2022).

Modern approaches in microbial characterization, including statistical and computational methods such as the Euclidean distance proximity matrix, have enabled more precise assessments of strain-level differences and similarities among Lactobacillus species (Olodu et al., 2024; Petrović et al., 2012). This method provides insights into phenotypic and genetic relationships, thereby contributing to a better understanding of each strain's probiotic efficacy and safety profile (Rapoo et al., 2023; Suleiman et al., 2022). Proximity matrix analysis has proven particularly effective in identifying candidate strains with the potential for targeted health benefits, advancing the development of LAB-based functional foods and nutraceuticals (Gizachew et al., 2023; Gizachew et al., 2013).

In this study, Lactobacillus strains isolated from Nigerian fermented cassava and corn products were characterized for probiotic properties, antimicrobial resistance, and phylogenetic proximity through Euclidean distance analysis (Saeed et al., 2023; Samuel et al., 2019). These findings contribute to the growing body of knowledge on LAB, supporting the safety and health-promoting potential of these indigenous strains, and highlighting their application in functional food innovation (Kaushik et al., 2009; Khushboo et al., 2023; Obafemi et al., 2022).

2. Material and Methods

The methodology comprised several key stages, including sample collection, isolation of bacterial strains, characterization, and evaluation of antimicrobial properties.

2.1. Sample Collection

Fermented cassava and maize were collected from ten different locations which include: Ikpoba hill area, Ekiosa market, New Benin market, Okha area, Uselu area, Oliha area, Oka market, Isiyor area, Ugbighoko market, and Aduwawa market. A total of 100 samples were collected from ten (10) different location with fifty (50) samples each for ground fermented cassava and fermented maize for a period of one month between August and September, 2023. The samples were obtained in foil paper, maintained in their original packaging, tagged, and delivered right away to the microbiology lab at Benson Idahosa University for analysis.

2.2. Fermentation Conditions

Fermentation of the slurry was performed in a batch by using 500ml fermenting pot filled to 450mL of distilled water. Cassava tubers were processed which include washing, peeling, chopping, and re-washing with water. The chopped or sliced cassava tubers were allowed to ferment for four days at room temperature in fermenting pots filled with sterile distilled water. Additionally, some maize grains (corn) were fermented for four days at room temperature in a fermenting pot filled with sterile distilled water. To a fineness of 0.05um, the fermented maize grains (corn) were ground. The cassava and corn flour slurry were rinsed and sample are obtained in sterile wide-mouthed jars, maintained in their original pack tagged and delivered right away to the microbiology lab at Benson Idahosa University for analysis.

2.3. Isolation of *Lactobacillus* sp. from Fermented Cassava and Corn/Maize

The isolation of the Lactobacillus spp. on de ManRogosa-Sharpe Agar (MRS; Oxoid, Cambridge, UK), was performed according to the method described by Olodu et al., (2024) and Drissi et al., (2015). 45 mL of sterile peptone water were used to homogenize 5 g of each sample. 1 ml of the homogenized sample was serially diluted in 9 ml of peptone water and 1ml of the solution was plated in MRS Agar. Triplicate plates were inoculated using the pour plate method and incubated at 37 °C for 48 hours in anaerobic jar 5–10% CO2. A morphologically unique, well-isolated colony was included in each sample, and it was chosen and streaked onto fresh MRS plates until a pure culture was obtained. The bacterial cells were suspended in MRS Agar in order to maintain pure cultures for use in subsequent studies (Olodu et al., 2024).

2.4. Identification of *Lactobacillus* sp.

The bacteria isolates were identified using their cultural, morphological and biochemical characterizations from fresh cultures of Lactobacillus spp. (isolates grown on MRS Agar for 24 hours) with modified versions of the techniques published by Olodu et al., (2024) and Drissi et al., (2015). The following tests were carried out; Gram staining, Motility, Catalase, Oxidase, Coagulation, Citrate, Indole Endospore, Hydrogen sulphide production, Gas production from glucose fermentation and Sugar fermentation.

2.4.1. Gram Staining

The test organism was smeared onto a spotless glass slide. After that, a drop of distilled water was added to moisten it. Additionally, it was placed over a flame to heat fix without burning the smear. Furthermore, 0.3% w/y crystal violet stain was added, and it was left to stand for a minute. The strain was dissolved using distilled water. After adding iodine (0.4% w/y) as a mordant and letting it stand for a minute, the mixture was cleaned with distilled water. After adding acetone/ethanol (95% w/y), a decolorizer was added and left for 10 seconds. The mixture was then quickly rinsed with distilled water, and safranin (0.4% w/y), the secondary stain, was added and left for one minute and this was cleaned with distilled water and left to dry. Next, using an oil immersion lens magnification of x100, the stained smear was examined under a microscope. Gram positive bacteria retain purple background while Gram negative bacteria retain pink or red colour (Joghataei et al., 2019; Kaur et al., 2022).

2.4.2. Motility Test

To determine whether the isolates were motile or non-motile species, the hanging drop method was used to conduct the motility test. First, a cleaned cover glass was used to hold a tiny drop of MRS broth containing a specific isolate. Next, a concave depression slide with its cavity facing downward onto the broth drop was adhered to the cover glass using Vaseline. The slide was then inverted and focus with the lowest magnification to the higer magnification using oil immersion to observe the motility. It was observed that the motile organism moved from one point to another, while the non-motile organism remained stationary (Adesulu-Dahunsi et al., 2022; Drissi et al., 2014).

2.4.3. Catalase Test

This test is used to identify organism that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. On a microscopic slide, a loopful of bacteria was mixed with a drop of 3% (v/v) hydrogen peroxide to assess the generation of oxygen bubbles. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The absence of bubbles indicates negative result (Gizachew et al., 2023; Gizachew et al., 2013).

2.4.4. Oxidase Test

The oxidase test was used to detect the presence of a cytochrome oxidase system that will catalase the transport of electrons between electron donors in the bacteria and a redox dye-tetrmethyl-p-phenylenediamine. The test uses reagents such as impregnated disks and N,N,N′,N′-tetramethyl-p-phenylenediamine, TMPD (or N,N-dimethyl-p-phenylenediamine, DMPD, which is also a redox indicator). A sterile swab was used to obtain a small amount of the isolate; a drop of oxidase reagent will be placed onto the culture, positive reaction turn the bacteria violet to purple immediately or within 10 to 30 seconds, absence of a violet to purple colour will be regarded as a negative result (Akter et al., 2023; Assohoun et al., 2023).

2.4.5. Coagulase Test

The coagulase test was used to determine the bacterium's ability to produce the enzyme coagulase, which causes blood plasma to clot. A bacterial colony was emulsified in a drop of water with minimal spreading. A drop of plasma was then added to this mixture. A positive result is indicated by the formation

of visible clumps or particles on a glass slide, while a negative result shows no clumping (Rapoo et al., 2023).

2.4.6. Citrate Test

The citrate test determines an organism's ability to utilize citrate as its sole carbon and energy source. This test is part of the IMViC series, commonly used to differentiate bacterial families, including Lactobacillaceae. After preparing citrate agar, 5 milliliters were dispensed into each test tube, autoclaved, tilted, and allowed to solidify. The test organism was then inoculated, and the tubes were incubated for 24 hours. A positive result is indicated by a color change from green to blue, while no color change indicates a negative result (Adesulu-Dahunsi et al., 2022; Drissi et al., 2014).

2.4.7. Indole Test

The indole test evaluates an organism's capacity to synthesize indole by utilizing tryptophan. As a component of the IMViC test, the test is used to distinguish between members of the Lactobacillaceae family. The ability of the organism to oxidize tryptophan and produce indole, pyruvic acid, and ammonia is tested by measuring indole production. By adding Kovac's reagent (dimethyl aminobenzaldehyde), the production of indole during the reaction can be detected. A positive result is indicated by the presence of a cherry-red layer at the top of the test tube; a negative result is indicated by its absence. A few drops of Kovac's reagent were added to an overnight peptone broth culture of the isolate. The formation of a cherry-red layer on the broth's surface indicated the production of indole (Akter et al., 2023; Assohoun et al., 2023).

2.4.8. Sugar Fermantative Test

Five (5) sugar was utilized; glucose, maltose, fructose, lactose and sucrose. The isolates were inoculated into the respective cooled sterilized tubes containing 10 ml of the basal medium (Peptone water), inverted Durham tube to detect gas production, 1ml of the 1% sugar solution (sterilized by steam sterilization for 30 min) and methyl red indicator. The inoculated tubes were incubated anaerobically at 370C for 48 hours. After incubation, the tubes were observed for acid and gas production by the respective isolate. Acid production was indicated by the formation of a yellow colour while gas evolution was determined by the formation of bubbles and spaces within the inverted Durham tube (Akter et al., 2023; Assohoun et al., 2023).

2.4.9. Endospore

The examination of spore production was used to categorize and identify bacteria as either spore formers or non-spore formers. On a microscopic slide, a bacterial smear was produced and heat fix without burning the smear, safranin and malachite green were colored for two minutes and thirty seconds. After rinsing with water to remove any remaining stain, the slide was examined under an oil immersion microscope. Spore forming bacteria retained green background colour and nonspore forming bacteria retained blue backgroyund (Assohoun et al., 2023).

2.4.10. Hydrogen Sulfide Production

Bacteria capable of sulfur reduction can produce hydrogen sulfide (H_2S). A single colony from each isolate was inoculated by stabbing into Triple Sugar Iron Agar (TSI) slants (HiMedia, M0211), prepared according to the manufacturer's instructions. The

tubes were incubated at 37°C for 24 hours in duplicates, with a non-inoculated tube serving as the negative control. Hydrogen sulfide production was detected by the formation of a black precipitate, indicating a positive result (Khushboo et al., 2023; Obafemi et al., 2022).

2.4.11. Gas Production from Glucose Fermantation

Isolates were classified as homofermenters, heterofermenters, or facultative heterofermenters based on their ability to ferment glucose. Each isolate was inoculated into centrifuge tubes containing modified MRS broth (prepared with 20 g of glucose per liter), with inverted Durham tubes placed inside. The tubes were incubated at 37°C for 24 hours in duplicates. Escherichia coli was used as a positive control, while a non-inoculated tube served as a negative control. Gas production was observed as bubbles trapped in the Durham tubes (Kaushik et al., 2009; Obafemi et al., 2022).

2.5. Isolation of Bacterial Strains

The isolation of *Lactobacillus* species was carried out using selective culture media to ensure the growth of target bacteria while minimizing the proliferation of unwanted microorganisms. Approximately 10 grams of each fermented sample were carefully weighed and homogenized in 90 mL of sterile saline solution to create an initial suspension. This suspension was then subjected to serial dilution using a ten-fold dilution technique to achieve concentrations suitable for microbial isolation. From each dilution, 0.1 mL aliquots were aseptically transferred and evenly spread onto de Man, Rogosa, and Sharpe (MRS) agar plates, a selective medium specifically formulated to support the growth of *Lactobacillus* species. The inoculated plates were incubated anaerobically at a temperature of 37°C for 48 hours using an anaerobic jar with gas packs to create an oxygen-free environment conducive to the growth of facultative anaerobes. After the incubation period, colonies displaying characteristic morphological features of *Lactobacillus*, such as circular, smooth, convex colonies with creamy white appearance, were carefully selected. These colonies were subsequently subcultured onto fresh MRS agar plates to ensure purity and eliminate potential contaminants. The purified isolates were then preserved under appropriate storage conditions for further biochemical and molecular identification to confirm their classification as *Lactobacillus* species.

2.6. Determination of antimicrobial properties of the isolates

The antimicrobial qualities of the isolates were ascertained in duplicate using the agar-well diffusion experiment. First, isolates were grown in MHA (Mueller-Hinton Agar) as a growth medium and incubated for 24 hours at 37 °C to create McFarland 0.5 standard solutions. The species from the isolates included Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus. Two milliliters of each pathogen culture (Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Citrobacter spp, Klebsiella pneumonia, and Escherichia coli) made using McFarland 0.5 standard solutions were pipetted out, flood inoculated onto MHA plates (90 mm in diameter), and then the plate was rotated to distribute

the inoculum. After that, the wells on the MHA plate were made using sterile, 9-mm cork borers. Following that, 180 µL of pH-adjusted cell-free isolate supernatants were added to each well. This was accomplished by centrifuging the MRS broth, which contained bacteria that had been cultured for a full day, for ten minutes at 10,000 g. Finally, the plates were incubated at 37 °C for 24 hours, and the zone of inhibition in each well was assessed. Inhibitions with diameter zones larger than 1 mm were considered to have strong antibacterial activity, according to Kaushik et al., 2009; Khushboo et al., 2023; Obafemi et al., 2022.

2.7. Pathogens Testing

Seven pathogenic organisms were collected from Microbiology laboratory in Central hospital, Benin City, Nigeria. These pathogens were cultured on different Agar and show different colouration and structures Candida sp were cultured on Sabouraud dextrose Agar which indicated white colour, Staphyloccocus and Streptococcocus sp were cultured on Chocolate Agar which indicated golden yellow, Pseudomonas sp were plated on Cled Agar which indicated greenish colonies, Salmonella species were plated on Salmonella Shigella Agar which indicated black colony, Escherichia coli were plated on MacConkey Agar which indicate pinkish colony and Klebsiella sp were plated on MacConkey Agar which indicated pink mucoid. Confirmation test was done using biochemical methods as follows; Staphylococcus species indicated catalase positive and coagulate positive, Streptococcus shows catalase negative, Salmonella species shows indole, citrate and urease negative. Eschericha coli shows indole positive. Candida species was incubated in serum at 37 °C for 2 - 4 hrs and then observed for the presence of tube structures indicated positive result, Klebsiella species shows a negative result in methyl-red test, indole test and catalase tests positive, Pseudomonas sp shows methy red negative and catalase positive. This is a simple, rapid, and economical identification technique with 98% sensitivity (Obafemi et al., 2022).

2.8. Statistical Analysis

The data obtained from the study were analyzed using descriptive and inferential statistical tools available in SPSS software version 23. Descriptive statistics, including mean, standard deviation, and frequency distributions, were used to summarize and represent the data effectively. The antimicrobial activity results were analyzed using a Euclidean distance proximity matrix to evaluate the degree of similarity and dissimilarity among the isolated *Lactobacillus* strains based on their inhibitory effects against test pathogens. Furthermore, one-way analysis of variance (ANOVA) was employed to determine significant differences in antimicrobial activity among the strains. A significance level of $p < 0.05$ was considered statistically significant, ensuring that observed differences were unlikely to have occurred by chance. All results were presented in tables for clarity and ease of interpretation.

3. Results

Table 1. Morphological and Biochemical Characteristics of *Lactobacillus* species Isolated from fermented Cassava and Corn

Colonial Morphology					**Staining Reactions**		**Biochemical Tests**							**Sugar Fermentation**						
Isolate code	**Shape**	**Cell shape**	**Cell arrangement**	**Colour**	**Gram staining**	**Spore staining**	**Catalase test**	**Indole production**	**Oxidase Test**	**Indole Test**	**CO_2 from glucose**	**Coagulase test**	**H_2S production**	**Fructose**	**Sucrose**	**Galactose**	**Lactose**	**Glucose**	**Citrate utilization**	**Probable isolates**
M1	CS	R	FL	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+veG	+veG	+ve	+veG	+veG	+ve	*Lactobacillus fermentum*
M2	CS	SR	C	WH	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+ve	+ve	+ ve	+ve	+ ve	-ve	*Lactobacillus ghanensis*
N1	CS	PP	S	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+veG	+veG	+ ve	+veG	+ve	-ve	*Lactobacillusdelbrueckii*
N2	BS	R	C	WH	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+ve	+ ve	+ ve	+ve	+ ve	-ve	*Lactococcus lactis*
N3	IS	R	C	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	+ ve	+veG	+veG	+ ve	+veG	+veG	-ve	*Lactobacillus reuteri*
S1	CS	SR	C	WH	+veG	- ve	-ve	-ve	-ve	+ve	+ve	- ve	-ve	+veG	+ ve	+ ve	+ ve	+ve	-ve	*Lactobacillus plantarum*
S2	BS	R	C	DC	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	-ve	+ve	+ ve	+ve	+ ve	-ve	*Lysinibacillus sphaericus*
S3	CS	SR	FL	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+veG	+veG	+ve	+veG	+veG	-ve	*Bacillus cereus*
S4	CS	R	FL	BC	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+veG	-ve	+ ve	+ve	+ve	-ve	*Bacillus pacificus*

SR=Single rods R=Rods PP=Paired rods C=Cocci in bunch FL=Filamentous CR=Creamy WH=Entire whitish A = Colour change +ve = Positive -ve = Negative, +veG=Gram positive -veG=Gram negative BC=Bright creamy DC=Dull creamy CS=Curved shape IS=Irregular shape BS=Broad shape

Table 2. Organisms Isolated from Fermented Cassava and Corn Samples from the Various Locations in Benin City, Nigeria (Based on Biochemical Test)

Location	Number of Samples	Number with Isolated Organisms	Isolates
Ikpobahill area	8	8(88.89%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus.*
Ekiosa market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
New Benin area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Okha area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Uselu area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Oliha area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Oka market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Isiyor area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Ugbighoko market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Aduwawa market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Total	90	89(98.89%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*

Table 3. Percentage Distribution of Bacterial Species Isolated from Fermented Cassava and Corn Sample from Benin City, Based on Biochemical Test

Location	*LACTOBACILLUS* ISOLATES									
	Lactobacillus fermentum	***Lactobacillus ghanensis***	***Lactobacillus delbrueckii***	***Lactobacillus plantarum***	***Lactococcus lactis***	***Lactobacillus reuteri***	***Lysinibacillus sphaericus***	***Bacillus cereus***	***Bacillus pacificus***	**Total Organism**
Ikpobahill area	1	1	-	2	-	1	-	1	2	
Ekiosa market	1	1	1	2	1	1	-	-	1	8
New Benin area	1	-	1	1	1	1	1	1	-	7
Okha area	1	1	2	1	1	-	-	-	1	7
Uselu area	2	1	1	1	1	1	-	1	2	10
Oliha area	1	2	1	1	1	1	1	1	1	10
Oka market	2	-	2	1	1	1	1	1	1	10
Isiyor area	1	1	-	2	1	-	2	2	1	10
Ugbighoko market	2	1	1	2	1	2	1	1	-	11
Aduwawa market	1	1	1	1	1	-	1	1	2	9
	13(14.4%)	**9(10%)**	**10(11.1%)**	**14(15.6%)**	**9(10%)**	**8(8.9%)**	**7(7.8%)**	**9(10%)**	**11(12.2%)**	**90(100%)**

Table 4. Antimicrobial Activity of *Lactobacillus* spp. and *Bacillus* spp. isolated from fermented Cassava and corn against Pathogenic bacterial isolates

	Clinical Pathogenic Bacterial isolates from Central Hospital, Benin City (Mean±SD), Zone of inhibition (mm)							
S/N	***Lactobacillus* sp.**	***Candida* sp.**	***Staphylococcus* sp.**	***Pseudomonas* sp.**	***Klebsiella* sp.**	***Escherichia coli***	***Salmonella* sp.**	***Streptococcus* sp.**
1	*Lactobacillus fermentum*	14.2±1.01	13.5±2.50	12.4±2.50	15.2±0.16	13.1±1.20	13.5±0.50	11.0±0.02
2	*Lactobacillus ghanensis*	11.5±1.50	11.6±0.80	12.0±0.40	11.5±0.50	10.0±0.20	9.0±0.70	10.0±0.30
3	*Lactobacillus delbrueckii*	9.0±0.60	9.0±0.50	10.0±0.80	9.0±1.40	11.0±1.30	9.5±2.10	7.0±0.60
4	*Lactobacillus plantarum*	15.0±0.16	16.5±0.25	17.8±0.50	18.1±0.10	14.8±0.50	17.5±0.80	8.0±0.90
5	*Lactococcus lactis*	9.0±1.30	10.4±0.30	8.0±1.60	8.0±0.15	9.0±0.16	9.0±0.16	7.0±0.90
6	*Lactobacillus reuteri*	11.0±0.80	10±1.20	10.5±0.12	12.4±0.22	9.0±1.80	11.2±0.50	9.0±0.12
7	*Lysinibacillus sphaericus*	10.5±0.40	8.5±1.50	9.0±1.10	9.0±0.15	12.0±0.25	11.2±0.80	9.0±0.10
8	*Bacillus cereus*	8.6±1.50	9.0±0.50	8.0±0.13	7.0±0.85	6.0±0.50	9.5.0±0.88	9.0±0.11
9	*Bacillus pacificus*	10.0±0.24	12.0±1.02	14.0±1.10	13.5±0.90	11.2±2.10	12.8.0±1.54	7.0±0.35

Table 5. Paired Samples Statistics

Paired Samples		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Staphylococcus sp.	*11.167*	*9*	*2.5792*	*0.8597*
Pair 2	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Pseudomonas sp.	*11.300*	*9*	*3.1749*	*1.0583*
Pair 3	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Klebsiella sp.	*11.522*	*9*	*3.6581*	*1.2194*
Pair 4	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 5	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 6	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Pseudomonas sp.	*11.300*	*9*	*3.1749*	*1.0583*
Pair 7	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Klebsiella sp.	*11.522*	*9*	*3.6581*	*1.2194*
Pair 8	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 9	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 10	*Pseudomonas sp.*	*11.300*	*9*	*3.1749*	*1.0583*
	Klebsiella sp.	*11.522*	*9*	*3.6581*	*1.2194*
Pair 11	*Pseudomonas sp.*	*11.300*	*9*	*3.1749*	*1.0583*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 12	*Pseudomonas sp.*	*11.300*	*9*	*3.1749*	*1.0583*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 13	*Klebsiella sp.*	*11.522*	*9*	*3.6581*	*1.2194*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 14	*Klebsiella sp.*	*11.522*	*9*	*3.6581*	*1.2194*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 15	*Escherichia coli*	*10.678*	*9*	*2.5728*	*0.8576*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*

Table 6. Paired Samples Correlations

Paired Samples		N	Correlation	Sig.
Pair 1	*Candida* sp. & *Staphylococcus* sp.	9	0.864	0.003
Pair 2	*Candida* sp. & *Pseudomonas* sp.	9	0.786	0.012
Pair 3	*Candida* sp. & *Klebsiella* sp.	9	0.905	0.001
Pair 4	*Candida* sp. & *Escherichia coli*	9	0.782	0.013
Pair 5	*Candida* sp. & *Streptococcus* sp.	9	0.494	0.177
Pair 6	*Staphylococcus* sp. & *Pseudomonas* sp.	9	0.906	0.001
Pair 7	*Staphylococcus* sp. & *Klebsiella* sp.	9	0.914	0.001
Pair 8	*Staphylococcus* sp. & *Escherichia* coli	9	0.696	0.037
Pair 9	*Staphylococcus* sp. & *Streptococcus* sp.	9	0.135	0.729
Pair 10	*Pseudomonas* sp. & *Klebsiella* sp.	9	0.937	0.000
Pair 11	*Pseudomonas* sp. & *Escherichia coli*	9	0.754	0.019
Pair 12	*Pseudomonas* sp. & *Streptococcus* sp.	9	0.006	0.989
Pair 13	*Klebsiella* sp. & *Escherichia coli*	9	0.777	0.014
Pair 14	*Klebsiella* sp. & *Streptococcus* sp.	9	0.213	0.582
Pair 15	*Escherichia coli* & *Streptococcus* sp.	9	0.058	0.881

Table 7. Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*Candida* sp. - *Staphylococcus* sp.	-0.1889	1.3014	0.4338	-1.1892	0.8114	-0.435	8	0.675
Pair 2	*Candida* sp. - *Pseudomonas* sp.	-0.3222	1.9766	0.6589	-1.8416	1.1971	-0.489	8	0.638
Pair 3	*Candida* sp. - *Klebsiella* sp.	-0.5444	1.8696	0.6232	-1.9815	0.8926	-0.874	8	0.408
Pair 4	*Candida* sp. - *Escherichia coli*	0.3000	1.6256	0.5419	-0.9495	1.5495	0.554	8	0.595
Pair 5	*Candida* sp. - *Streptococcus* sp.	2.4222	2.0030	0.6677	0.8826	3.9619	3.628	8	0.007
Pair 6	*Staphylococcus* sp. - *Pseudomonas* sp.	-0.1333	1.3766	0.4589	-1.1915	0.9248	-0.291	8	0.779
Pair 7	*Staphylococcus* sp. - *Klebsiella* sp.	-0.3556	1.6712	0.5571	-1.6401	0.9290	-0.638	8	0.541
Pair 8	*Staphylococcus* sp. - *Escherichia coli*	0.4889	2.0096	0.6699	-1.0558	2.0336	0.730	8	0.486
Pair 9	*Staphylococcus* sp. - *Streptococcus* sp.	2.6111	2.7728	0.9243	0.4797	4.7425	2.825	8	0.022
Pair 10	*Pseudomonas* sp. - *Klebsiella* sp.	-0.2222	1.3036	0.4345	-1.2243	0.7798	-0.511	8	0.623
Pair 11	*Pseudomonas* sp. - *Escherichia coli*	0.6222	2.0945	0.6982	-0.9878	2.2322	0.891	8	0.399
Pair 12	*Pseudomonas* sp. - *Streptococcus* sp.	2.7444	3.4724	1.1575	0.0753	5.4136	2.371	8	0.045
Pair 13	*Klebsiella* sp. - *Escherichia coli*	0.8444	2.3179	0.7726	-0.9373	2.6262	1.093	8	0.306
Pair 14	*Klebsiella* sp. - *Streptococcus* sp.	2.9667	3.6315	1.2105	0.1753	5.7581	2.451	8	0.040
Pair 15	*Escherichia coli* - *Streptococcus* sp.	2.1222	2.8670	0.9557	-0.0815	4.3260	2.221	8	0.057

Table 8. Proximity Matrix (Euclidean Distance) of Antimicrobial Activity of *Lactobacillus* sp from Fermented Cassava and Corn

	Euclidean Distance								
	Lactobacillus fermentum	***Lactobacillus ghanensis***	***Lactobacillus delbrueckii***	***Lactobacillus plantarum***	***Lactococcus lactis***	***Lactobacillus reuteri***	***Lysinibacillus sphaericus***	***Bacillus cereus***	***Bacillus pacificus***
Lactobacillus fermentum	0.000	5.946	10.578	7.688	11.860	7.399	9.690	13.879	6.704
Lactobacillus ghanensis	5.946	0.000	5.767	11.853	6.778	2.805	5.555	8.272	4.566
Lactobacillus delbrueckii	10.578	5.767	0.000	15.854	3.311	4.981	2.915	6.096	6.804
Lactobacillus plantarum	7.688	11.853	15.854	0.000	17.490	13.367	15.917	19.872	9.737
Lactococcus lactis	11.860	6.778	3.311	17.490	0.000	5.811	4.567	4.015	8.640
Lactobacillus reuteri	7.399	2.805	4.981	13.367	5.811	0.000	5.031	7.153	5.225
Lysinibacillus sphaericus	9.690	5.555	2.915	15.917	4.567	5.031	0.000	6.698	7.899
Bacillus cereus	13.879	8.272	6.096	19.872	4.015	7.153	6.698	0.000	10.966
Bacillus pacificus	6.704	4.566	6.804	9.737	8.640	5.225	7.899	10.966	0.000

This is a dissimilarity matrix

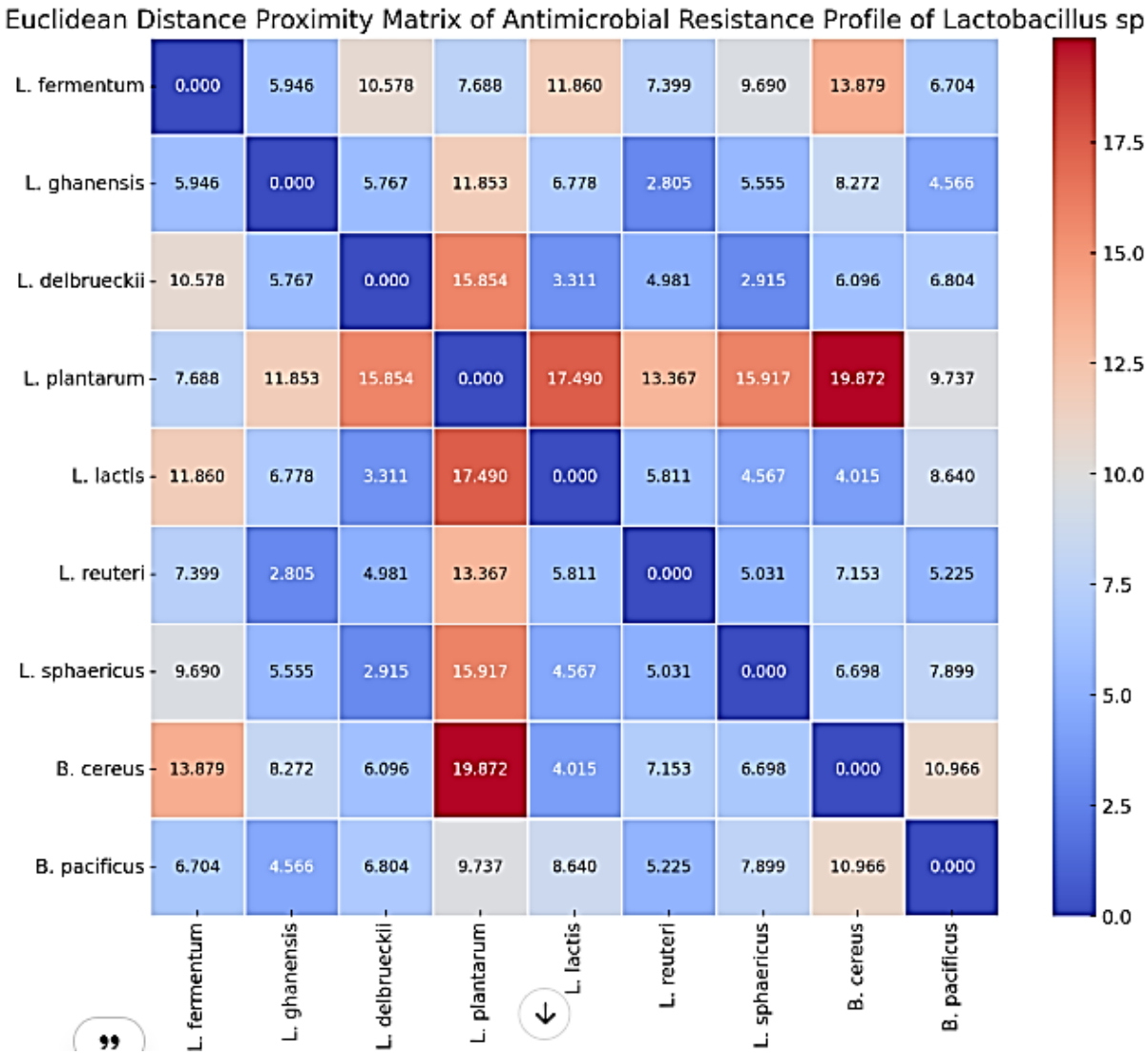

Figure 1. The Euclidean distance heatmap for the antimicrobial resistance profile of *Lactobacillus* species from fermented cassava and corn. The heatmap visually represents the degree of dissimilarity between the bacterial strains, with color intensity indicating the distance values

4. Discussion

The antimicrobial activity of *Lactobacillus* species from fermented cassava and corn against clinical isolates (*Candida* sp., *Salmonella* sp., *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Escherichia coli*) demonstrated varied inhibition zones. Notably, *Lactobacillus fermentum* exhibited significant inhibition zones, ranging from 11.0 ± 0.20 mm to 15.2 ± 0.16 mm. Similarly, *Lactobacillus plantarum* displayed the highest inhibition zones, with values reaching up to 18.1 ± 0.10 mm against *Streptococcus* sp. and 17.8 ± 0.50 mm against *Staphylococcus* sp. These findings align with previous studies that reported the robust antimicrobial activity of *Lactobacillus plantarum* against gram-positive and gram-negative bacteria (Kaushik et al., 2009; Adesulu-Dahunsi et al., 2022). In contrast, *Bacillus cereus* exhibited relatively lower inhibition zones, with values of 6.0 ± 0.50 mm against *Pseudomonas* sp. and 8.6 ± 1.50 mm against *Candida* sp, Similarly, findings by Akter et al. (2023) revealed that *Lysinibacillus sphaericus* demonstrated limited efficacy, with inhibition zones ranging between 6.0 ± 0.50 mm to 9.5 ± 0.88 mm. Their results compared favourably with the results obtained in this study.

Statistical analysis presented in Table 5 revealed significant variations in inhibition zones across pathogen pairs. For instance, *Candida* sp. had a mean inhibition zone of 10.978 mm, while *Staphylococcus* sp. recorded a mean of 11.167 mm. The minimal difference of 0.1889 mm in Table 7 suggests consistent inhibitory responses by certain *Lactobacillus* strains (Gizachew et al., 2023; Joghataei et al., 2019). Correlation analysis in Table 6 revealed a strong positive relationship (r=0.906, p = 0.001) between inhibition responses of *Staphylococcus* sp. and *Pseudomonas* sp., supporting the hypothesis of shared inhibitory mechanisms, potentially involving bacteriocins and lactic acid production (Dahiya & Nigam, 2023; Dahiya & Nigam, 2022).

Statistical differences further highlighted unique responses, particularly between *Streptococcus* sp. and *Candida* sp. (p = 0.007), indicating selective pathogen-targeting abilities of specific *Lactobacillus* strains. These findings are consistent with earlier studies by Aderolake et al. (2023) and Owusu-Kwarteng et al. (2015), emphasizing the potential of *Lactobacillus fermentum* and *Lactobacillus plantarum* as effective probiotics for therapeutic applications.

The Euclidean Distance Matrix (Table 8) categorized antimicrobial profiles based on proximity, with the smallest dissimilarity (6.704 mm) observed between *Lactobacillus fermentum* and *Bacillus pacificus*, while the highest dissimilarity (19.872 mm) was recorded between *Lactobacillus plantarum* and *Bacillus cereus*. Figure 1 visually reinforces these findings, with lower distances indicating shared inhibitory mechanisms. These results align with reports by Khushboo et al. (2023) and Assohoun et al. (2023), underscoring the importance of strain-specific antimicrobial efficacy in probiotic formulation.

In summary, *Lactobacillus fermentum* and *Lactobacillus plantarum* demonstrated superior antimicrobial activity, making them promising candidates for probiotic therapies. Conversely, the

limited inhibitory effects of *Bacillus cereus* and *Lysinibacillus sphaericus* suggest a reduced therapeutic potential. These findings align with previous literature (Mokoena et al., 2016; Akter et al., 2023) and highlight the need for further strain characterization to optimize their use in targeted antimicrobial applications.

5. Conclusion

In conclusion, the Lactobacillus strains isolated from fermented cassava and corn exhibit significant probiotic potential, characterized by notable antimicrobial activity against pathogenic bacteria and robust antioxidant properties. The study's findings highlight the strains' resilience in harsh conditions, reinforcing their suitability for application in functional food products. The uniformity in antimicrobial effectiveness, as indicated by the Euclidean distance proximity matrix, suggests that these isolates could serve as effective probiotics in the food industry. Overall, this research contributes valuable insights into the development of sustainable, plant-based probiotics, enhancing both food safety and consumer health.

Ethical Statement

The study is proper with ethical standards, it was approved by the Department of Biological Sciences (Microbiology), Benson Idahosa University on 26th February, 2024. The Ethics Committee approval number is ETH/2024/0026.

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Presentation Information

The data from this study were presented as an oral presentation at the Department of Biological Sciences (Microbiology), Benson Idahosa University

Conflicts of Interest

The authors declare no conflicts of interest regarding this study.

Author Contributions

Blessing Adoh Olodu contributed to the study design, data collection, laboratory analysis, statistical analysis, and manuscript drafting. Prof. Stephen Amadin Enabulele provided guidance on study design, supervised the laboratory work, contributed to data interpretation, and critically revised the manuscript for important intellectual content.

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Review

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Plant-Derived Exosome-Like Nanoparticles Based Treatments In Cancer Therapy

Gaye Umurhan[1,2*], Meriç Arda Eşmekaya[2], Burhan Ertekin[2,3], Arın Tomruk[2]

[1]Gazi University, Non-Ionizing Radiation Protection Application and Research Center

[2] Gazi University, Medical Faculty, Department of Biophysics

[3]Tokat Gaziosmanpasa University, Medical Faculty, Department of Biophysics

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Abstract

Plant-derived exosome-like vesicles (PELVs) are nanometer-sized particles comprising proteins, lipids, nucleic acids, and small molecule substances generated from plants. PELVs have many advantages, such as low toxicity, efficient cellular uptake, high biocompatibility, stability, and large-scale production. PELVs can regulate intercellular communication by releasing their contents, including mRNA, miRNA, lipids, and proteins. Plant-derived exosome-like vesicles (PDELVs) have attracted considerable attention in scientific research owing to their promising therapeutic effects and researches have assessed the the extensive therapeutic potential of PDELVs in the treatment of various diseases including cancer treatment. They exhibit various clinical attributes and therapeutic benefits over conventional pharmaceuticals. This mini-review aims to summarize and categorize the main paths followed by scientists working with the PDELNs for cancer therapy.

Gaye Umurhan **(Corresponding author):** ORCID: 0000-0001-8891-9510. e-mail: gayesezgin@gazi.edu.tr
Meriç Arda Eşmekaya: ORCID: 0000-0003-0469-4954, e-mail: ardaesmekaya@gazi.edu.tr
Burhan Ertekin: ORCID: 0000-0003-2804-047X, e-mail: burhan.ertekin@gop.edu.tr
Arın Tomruk: ORCID: 0000-0002-7600-0811, e-mail: atomruk@gazi.edu.tr

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1. Introduction

Scientists have focused on nano-sized biological materials that are originated from human body to achieve a successful and effective treatment in the targeted cancer therapy. The studies conducted on cancer treatments showed that various structural and functional factors of these nano-sized materials affect the molecular systems: their synthesis, releases, sizes, and contents. It might be interesting that recent studies revealed that there are compositional similarities between plant and mammalian derivatives nano-sized vesicles. For this reason, scientists have turned to using the plant derivative tiny lipid bilayers for their medical treatments and therapeutics. Because of their high biocompability and non-toxicity to human, these plant derived exosome-like nanoparticles (PDELNs) can easily trigger the immune response and inflammation. These nanoparticles can participate in many physiological process to regenerate and control the human cancerous or inflammatory tissues (Zhang et al., 2021). Therefore, scientists have recently focused on PDELNs to identify and improve the usage area of these PDELNs vesicles in cancer therapy.

2. Content of PDELNs

Mammalian cell-derived exosomes have a lipid bilayer that is mostly composed of phosphotidylserine, ceramides, cholesterol, and glycosphingolipids, which give them stability and a special rigidity. On the other hand, PDELN membranes are abundant in digalactosyldiacylglycerol (DGDG), phosphatidic acid (PA), phosphatidylcholines (PC), and monogalactosyldiacylglycerol. These unique lipid characteristics offer inherent regulatory functions for mammalian cells. A study found that ginger-derived exosomes include 25-40% of total lipids as phosphatidic acid, 25-40% as galactosyldiacylglycerol, and 20-30% as monogalactosyldiacylglycerol. (Zhang et al., 2016). One phospholipid that distinguishes out among the others is phosphatidic acid, which is frequently present in PDELNs and helps target and stimulate the mTOR pathway, which is in charge of cell growth, proliferation, and repair. By establishing a cellular block in the cell membrane, phosphatidylcholines, a source of choline in the body, can shield a colon cell wall (Kim et al., 2022). The morphology and lipid composition of PDELNs are critical for intestinal cell absorption. PDELNs have large amounts of phospholipids, whereas mammalian exosomes are rich in cholesterol and sphingomyelin. (Suharta et al., 2021).

The regulation of glycolipid metabolism, membrane-associated proteins, and cytosolic proteins is controlled by the structural proteins of PDELNs vesicles. According to the literature, large number of confirmed protein types have been detected in PDELNs, such as heat shock proteins considered exosome markers (e.g. CD9 and CD63), transmembrane proteins (e.g. actin, proteolysis, aquaporin, and chloride channel proteins), defense proteins, and other plasmalemma-associated proteins. BKEBN obtained from bitter orange, grapefruit, lemon, and sweet orange was studied and shown to have a considerable amount of proteins (Sarasati et al., 2023, Sha et al., 2024).

Other significant discovery for exosomes is the microRNA (miRNA, 19–24 nt, non-coding RNAs) used for targeted drug delivery systems. MicroRNAs regulate the expression of numerous mRNAs, which is critical in many biological processes. They may also impact the course of certain illnesses by facilitating cell-to-cell communication. miRNAs are involved in all cellular processes and are critical for differentiation of cells and homeostasis. They are released into the extracellular fluid and can serve as biomarkers for the identification of various illnesses. (Asgarpour et al., 2020). miRNAs identified in PDELNs can control gene expression via guiding mRNA cleavage. A research study found that miR168a generated from rice can precisely target and regulate LDLRAP1 (low-density lipoprotein receptor adaptor protein 1) expression in mice livers. In another study, it was demonstrated that plant miR159 inhibits the growth of breast cancer cells by targeting the TCF7 gene. It is possible that the miRNA carried by PDELNs determines their functions. Additionally, miRNA patterns in PDELNs vary by plant species, implying that ELNs derived from diverse edible plant sources may serve different regulatory roles (Leng et al., 2024).

3. Isolation of PDELNs

Numerous methods for the isolation of exosomes in a significant quantity and purity have been developed thanks to the rapid advancements in science and technology. In order to facilitate exosome isolation, each method takes advantage of a specific characteristic of the particles, like shape, density, size, and surface proteins (Lie et al., 2017). Techniques for isolating exosomes, which are essential in biological research, have advanced significantly. Exosome isolation techniques are generally categorized as follows.

3.1. Ultracentrifugation

This method has established the gold standard for isolating and purifying ELNs, thanks to its simplicity and low cost. The technique relies on the variations in the size and density of the separated particles. Centrifugation is commonly used for the separation and purification of particulate materials, as well as to investigate the hydrodynamic properties of polymeric materials, comprising biopolymers like DNA and proteins. A suspension's particles are successively separated based on their physical characteristics as well as the solvent's density and viscosity, all of which are influenced by the centrifugal force that is applied.

Ultracentrifugation is expected to account for 80% of all exosome isolation techniques utilized in exosome research. Many people utilize ultracentrifugation as an isolation method because it is simple to use, requires little technical experience, is cost-effective over time (e.g., an ultracentrifuge for long-term usage), and requires little or no sample pretreatment. Because of these factors, researchers studying exosomes are increasingly choosing to use ultracentrifugation-based methods. (Li et al., 2017, Omrani et al., 2024, Miron et al., 2024).

3.2. Differential Ultracentrifuge

Differential ultracentrifugation is another centrifugation-based method. In summary, the exosome source, biological fluid or plant broth, is centrifuged at escalating speeds and durations to

remove larger and higher-density components; the resulting pellets are discarded. The supernatant is centrifuged at 100,000 × g or more to recover the exosome-containing pellet, which is subsequently resuspended and washed in a buffer. This method is frequently adapted for different exosome sources. (Akuma et al., 2019).

3.3. Density Gradient Centrifugation

Density gradient ultracentrifugation is now widely used to isolate extracellular vesicles such as exosomes. Exosomes are separated via density gradient ultracentrifugation based on their size, mass, and density in a centrifuge tube's pre-configured density gradient medium, where the density progressively drops from bottom to top. After a lengthy ultracentrifugation cycle, a sample is layered as a narrow band on top of the density gradient medium. Following the application of centrifugal force, the sample's solutes—including exosomes—migrate as distinct zones across the density gradient medium, each moving toward the bottom at its own rate of sedimentation. This results in the formation of distinct solute zones. The divided exosomes can then be simply retrieved using simple fraction. One of the drawbacks of density gradient ultracentrifugation, in contrast to differential ultracentrifugation, is that its capacity is mostly restricted to the narrow load zone (Zhang et al. 2018).

3.4. Size Exclusion Chromatography (SEC)

The method employs a porous gel filtration polymer as the stationary phase and the original biofluid as the mobile phase. Differential elution is possible due to the stationary phase's structure. Large particles are extracted first, then smaller vesicles, and finally proteins not bound to the membrane. Because larger particles can only pass through fewer pores, they travel shorter distances to the end of the column and are separated faster than their smaller counterparts. SEC separates small and big vesicles and removes pollution from soluble proteins that are not attached to exosomes. As a result, the exosome is pure, undamaged, and reasonably priced. Nevertheless, exosomes and identically sized microvesicles cannot be distinguished by SEC. (Sidhom et al., 2020).

3.5. Ultrafiltraiton

The current generation of commercial membrane filters makes it easier to isolate particles of a specific size due to their small pore size distribution and range of sizes. Micro- or ultrafiltration is frequently employed in conjunction with an ELN isolation approach by researchers. Ultrafiltration, in instance, can be utilized as a step between successive ultracentrifugation stages and gel filtration chromatography. However, only microfiltration and ultrafiltration are suitable for ELN isolation. (Konoshenko et al., 2018).

3.5.1. Precipitation

Following ultracentrifugation, the approach based on ELN precipitation in PEG (Polyethylene glycol) solutions appears to be the most popular. This technique exploits chemicals' decreased solubility in PEG solutions, which are superhydrophilic polymers. The technique involves combining the sample with the polymer solution, incubating it, then precipitating the ELNs using low-speed centrifugation (1500 ×g). The

PEG approach allows for the simultaneous processing of huge numbers of samples. The technique is simple, rapid, and scalable. (Konoshenko et al., 2021).

4. Characterization of PDELNS

After the purification of PDELNs vesicles, scientists must be characterized their biological materials depending on four categories: size, concentration, purity, and content.

4.1. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

With the capacity to identify tiny exosomes, transmission electron microscopy (TEM) is the most efficient method for researching the morphology and structure of exosomes. The negative staining method is simple and quick, taking only a few hours, and the TEM resolution is around 1 nm.

Scanning electron microscopy (SEM) uses a fine-pointed beam rather than a broad beam like TEM to analyze samples line by line. As a result, SEM concentrates on the surface of the materials, producing a three-dimensional image of the exosomes rather than the two-dimensional image obtained by TEM. (Dilsiz et al., 2024).

4.2. Nanoparticle Tracking Analysis (NTA)

Biophysical approaches are utilized to determine the exosomal size range. NTA, an optical particle tracking device, is one such biophysical method that can quantify the concentration and size distribution of exosomes in the 10 nm to 2 μm range. The exosomal motion path is identified to determine the particle speed. This approach tracks the Brownian motion of nanoparticles in a liquid suspension at the particle level. NTA analyzes exosome mobility by tracking each particle using image analysis. The motion can then be connected to particle size. The size, concentration, size distribution, and phenotypic of the particles can all be ascertained from the findings of this technique (Alzhrani et al., 2021 ; Gurunathan et al., 2019).

4.3. Atomic Force Microscopy (AFM)

Another technology used in the ELN characterization procedure is AFM (Atomic Force Microscopy). It enables the high-resolution analysis needed to investigate EV structure while allowing for the in situ assessment of label-free samples with minimal sample preparation. By detecting the contact between a probe tip and the sample surface, AFM can determine the size distribution, concentration, and form of EVs. (Gazze et al., 2021).

4.4. Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) is an optical analysis technique used to determine the size and distribution of submicron particles. It is impossible to display details regarding the amount or concentration of a certain particle type because DLS analyzes each particle in a sample instantly. DLS indicates a defined range for EV diameter, however it is difficult to identify the concentration. As a result, to finish categorizing EVs, DLS technology is generally used in conjunction with other technologies. (Wu et al., 2024).

4.5. Flow Cytometry

Flow cytometry is a technique that captures the fluorescence and scattering signals generated by individual particles passing through a flow chamber under the illumination of a laser beam.. Because of its capacity to analyze multiple characteristics at the same time, flow cytometry is one of the most commonly used technology for studying exosomes. The device can count particles larger than 500 nm, which is useful for detecting microvesicles and apoptotic bodies. Because of side detection limits, typical cytometers may overlook nanoparticles less than 300 nm. (Rupert et al., 2017; Kurian et al., 2021).

4.6. Zeta Potential Measurement

The measurement of zeta potential (ZP), a measure of colloidal stability, is influenced by the electrophoretic mobility and surface charge in a suspension. Nanoparticles that are electrically charged are found in dispersed systems including emulsions, suspensions, and colloidal dispersions. ZP regulates the net surface charge of the nanoparticles in these dispersed systems, as well as the stability of the interactions between the particles and the medium, including the propensity of the particles to aggregate. As a result, ZP is one of the most valuable techniques for studying nanoparticle collective behavior, including colloidal stability, as shown in extracellular vesicles in dispersed systems. Similarly, ZP is one of the strategies used to evaluate the activity of extracellular vesicles in biological reactions. (Midekessa et al., 2020). Higher values of the Zeta potential indicate a more stable system of smaller dispersed particles, whereas lower absolute values indicate a stronger propensity to coagulate or concentrate. (Zhu and He, 2023).

Extracellular vesicles have a multicomponent structure containing small molecules such as proteins, lipids and nucleic acids and various high molecular weight materials. Recent studies have used spectroscopic techniques such as Raman (Guerrini et al., 2021), LC-MS (Zhao et al., 2024), GC-MS (Irıawati et al., 2024) and FTIR (Soares Martins et al., 2020) to evaluate the chemical composition of exosomes.

5. Biological Effects of PDELNs on Different Cancer Types

Stanly et al. investigated the anticancer effects of *ELNs isolated from grapefruit* on the A375 human melanoma cell line. The study reported that EBNPs caused a pause in the G1 phase of the A375 cell cycle and inhibited cell proliferation (Stanly et al., 2020). Kim et al. found that ELNs obtained from carrots helped neutralize free radicals in the cell and effectively reduced oxidative stress in cardiomyoblast and neuroblastoma cells by increasing the activity of antioxidant enzymes (Kim et al., 2021).

Studies have investigated the effects of PELNs on various types of cancer. In one study, *lemon-derived ELNs* were shown to arrest the S phase of the cell cycle and induce apoptosis in stomach cancer (Yang et al., 2021), while they were found to induce trace-mediated cell death in colon, blood, and liver cancer. They were also shown to mediate the inhibition of lipid metabolism and down-regulate Acetyl-CoA Carboxylase 1 in colon and blood cancer (Raimondo et al., 2018).

Kim et al. showed that *ELNs isolated from Panax ginseng* can effectively cross the blood-brain barrier, inhibit the growth and proliferation of glioma cells, and increase the apoptosis of tumor cells with both in vitro and in vivo studies (Kim et al., 2023). There are also studies in the literature on the effects of ginseng-derived ELNs on skin, colon, and breast cancer. ELNs were found to change macrophage polarization in skin cancer studies (Cao et al., 2019), and programmed cell death protein-1 monoclonal antibody and cold tumor environment were found to change in colon and breast cancer (Han et al., 2022).

The activity of *ELNs isolated from bitter melon* (BMELNs) in various cell lines has also been investigated. In one study, it was found that BMELNs inhibited the proliferation, migration, and invasion of glioblastoma cells by regulating the PI3K/AKT signaling pathway (Wang et al., 2022). Another study showed that BMELNs arrested the S-phase cell cycle of oral cancer, induced apoptosis, and reduced the resistance of cancer cells to 5-Fluorouracil by reducing NLRP3 expression (Yang et al., 2021).

In a study where *ELNs obtained from the leaves of the Gundelia munzuriensis* plant were applied to lung cancer (A549) and colon cancer (HCT116) cell lines, it was observed that they affected the viability of HCT 116 cancer cells by 40-50%, while a 50% decrease was observed in A549 cell lines (Demirhan et al., 2023). In another study aimed at investigating the cytotoxic effects of exosome-like nanoparticles (GdELN) obtained from the Gundelia Dersim plant native to Turkey on cell viability in human lung cancer (A549) and human colon cancer (HCT 116) cell lines, it was observed that isolated GdENs increased the concentrations of GdELNs that showed cytotoxic effect by reducing cell viability at different doses in HCT 116 cells (Erman et al., 2023).

Potesta et al., 2020 showed that *moringa oleifera-derived ELNs* induced apoptotic cell death in blood and cervical cancer cells. Ozkan et al. reported that garlic-derived ELNs caused caspase-mediated apoptosis in kidney and liver cancers (Ozkan et al., 2021). Another study found that corn-derived ELNs inhibited the proliferation of colon cancer cells and activated the release of tumor necrosis factor-α in macrophages (Sasaki et al., 2021).

It has been reported that *ELNs from Dendropanax morbifera* reduce cancer-associated fibroblasts around the tumor in skin cancer and prevent metastasis, while its use together with ELNs from Pinus densiflora has been reported to have anticancer effects in breast and skin cancer (Kim et al., 2020).

Liu et al. have shown that miR159 found in *soybean-derived ELNs* significantly reduces colon tumor formation in mice. These findings have shown that miR159 suppresses the expression of MYC oncogene in colon cancer cells in vitro using TCF-7-mediated signaling pathways (Liu et al., 2021).

It has been shown that hibiscus induces apoptosis in prostate cancer cells, especially in the LNCaP cell line. This effect has been observed in both in vitro and in vivo experiments. In animal experiments, specifically xenograft experiments in 8-week-old mice, mallow extract significantly inhibited tumor growth (Hui-Hsuan et al., 2012).

Ethical Statement

Ethics committee approval was deemed unnecessary for this study, given that open-access sources were utilized.

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Presentation Information

The findings of this study have not been presented at any conference or journal.

Conflicts of Interest

The authors declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

Author Contributions

The authors contribute as follows: Gaye Umurhan contributed to the literature search and editing of the report. Burhan Ertekin and Arın Tomruk revised the manuscript and Meriç Arda Eşmekaya conducted the supervision and final revision of the manuscript.

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Review

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Professional Identity Formation in Nursing: The Role of Travelbee's Human-to-Human Relationship Model

İnci İnceleme[1*], Satı Demir[2]

[1]Gazi University, Graduate School of Health Sciences, Ankara, Türkiye

[2] Gazi University, Department of Nursing, Ankara, Türkiye

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Abstract

Professional identity, which integrates personal values, ethical principles, and professional knowledge with the core concepts of nursing, is an essential aspect of nursing education, especially during the transition to professional practice. However, this development is often hindered by challenges such as low self-confidence, emotional fatigue, and communication barriers, particularly in clinical environments. Joyce Travelbee's Human-to-Human Relationship Model offers a comprehensive framework for addressing these challenges by emphasizing the significance of empathy, meaningful communication, and interpersonal connections in nursing care. The model delineates five distinct phases of the nurse-patient relationship—orijinal encounter, emerging identities, empathy, sympathy, and rapport—that promote deeper and more meaningful interactions with patients. This holistic, patient-centered approach enables nurses to address not only the physical health of patients but also their emotional and psychosocial needs, thereby fostering the development of a strong professional identity. This review underscores Travelbee's model as a transformative framework that bridges the technical and emotional dimensions of nursing practice. By cultivating a compassionate, human-centered approach to care, the model empowers both nursing students and professionals to build a robust professional identity. Future research should focus on developing effective strategies to integrate Travelbee's model into nursing education, ensuring its full potential in supporting professional identity formation is achieved.

İnci İnceleme **(Corresponding author);** ORCID: 0000 0002 9366 9365, e-mail: inciinceleme@gazi.edu.tr
Satı Demir; ORCID: 0000 0003 4232 8891, e-mail: satiunal@gazi.edu.tr

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1. Introduction

Professional identity in nursing is a conceptual structure that integrates the basic principles, values and understandings of the profession with the individual's personal values, attitudes and beliefs (Cowin et al., 2013; Philippa et al., 2021). Professional identity is not only limited to individual characteristics; it also includes knowledge, recognition by the community, group belonging, ethical rules and social control of professional behaviours (Fitzgerald, 2020; Willetts & Clarke, 2014). This structure is critical for nursing students to adopt their professional roles and maintain their professional attitudes throughout their professional lives (Cöplü & Kartin, 2019). However, professional identity development is a complex process and both the experiences of the individual in the education process and the difficulties encountered in the clinical environment can shape this process (Vabo et al., 2022; Yi et al., 2024).

Especially during nursing education, students' ability to develop their professional identities depends on the provision of an effective learning environment and the support of educators who guide students (Fitzgerald & Clukey, 2022; Vabo et al., 2022; Zeng et al., 2022). In this process, students form their professional ethical values (Haghighat et al., 2020), improve their communication skills with patients and the healthcare team, and gain knowledge and skills specific to their profession (Philippa et al., 2021).

However, it is known that nursing students face various difficulties in the process of professional identity development. Inadequate knowledge, deficient practical skills and underdeveloped communication competences, especially in the clinical learning environment, are among the main factors that negatively affect this process (Jamshidi et al., 2016). It is stated that these difficulties may lead to negative consequences such as lack of self-confidence (Vabo et al., 2022), professional role stress (Sun et al., 2016) and emotional burnout (Yi et al., 2024). These challenges may negatively affect not only the performance of students in the educational process, but also their long-term professional identity development. Nurses stated that long-term care and contact with patients caused them to experience psychological and emotional stress, and they could not communicate effectively with patients due to psychological tension. Moreover, it is stated that nurses believe that empathy is not given enough importance as a care value as a result of managers' approaches that prioritise physical care, and this leads to ignoring the psychological needs of patients (Taleghani et al., 2018). This suggests that nursing students should strengthen their professional identities by internalising not only technical skills but also values such as empathy, communication and patient-centred care. In this context, educational programmes that support professional identity development can contribute to nursing students' adoption of their professional roles and overcoming the difficulties they face in this process (Maginnis, 2018; Yi et al., 2024).

At this point, Travelbee's Human-to-Human Relationship Model emerges as a significant guide in supporting professional identity development by

highlighting the importance of empathy, meaningful communication, and interpersonal interactions within the nurse-patient relationship (Travelbee, 1969). The model is noted for encouraging nursing students to establish more authentic connections with patients and their families by emphasizing empathy, compassion, and trust (de Medeiros et al., 2022; Jang & Kim, 2019). This review aims to examine the role of Travelbee's Human-to-Human Relationship Model in fostering the professional identity development of nursing students.

2. The Role of Travelbee's Human-to-Human Relationship Model in Professional Identity Development

Joyce Travelbee's human-to-human relationship model is an important theoretical framework that supports the development of professional identity in nursing and focuses on nurse-patient interactions (Shelton, 2016). This model lays the foundation for a meaningful and effective care process by highlighting the central role of interpersonal relationships in nursing practice (de Medeiros et al., 2022; Travelbee, 1969). At the heart of Travelbee's model lies the principle that the nurse should view the patient not merely as a biological entity but also as an individual with emotional, social, and spiritual needs (Parola et al., 2020; Travelbee, 1969). Such a perspective supports nurses in perceiving their profession as a life purpose, making it an integral part of their professional identity.

Effective nursing care, according to this model, can only be achieved when a meaningful relationship is established between the nurse and the patient (Travelbee, 1969). This relationship is thought to strengthen nurses' professional identities by fostering a more conscious adoption of their professional roles.

Within Travelbee's model, elements such as empathy, voluntary interaction, professional responsibility awareness, and role clarity emerge as key factors for nurses in shaping their professional identities (Travelbee, 1969). Empathy, in particular, enhances nurses' dedication to their profession and motivates them to embrace the core principles of patient-centred care (Waird, 2023). Given that a lack of empathy can adversely impact both the quality of nursing care and patient outcomes (Burkhartzmeyer et al., 2021), Travelbee's empathy-focused approach offers valuable guidance in addressing such deficiencies. As a result, nurses can move beyond a purely technical approach and cultivate a holistic care perspective that prioritises patients' emotional and psychosocial needs. The ability to connect with patients not only on a physical health level but also by forming a human bond significantly influences the growth of their professional identities (Wang et al., 2023).

Voluntary interactions enable nurses to anticipate and address patient needs more effectively, transcending a task-oriented mindset (Fernndez-Basanta et al., 2023). In this regard, it is emphasised that viewing nursing solely as a job can limit the quality of patient care (Kago et al., 2024; Travelbee, 1969).

By fostering empathy and encouraging meaningful interactions, Travelbee's model guides nurses towards developing a deeper understanding of their roles and responsibilities, ultimately strengthening their professional identity (Travelbee, 1969). Travelbee highlights the critical role of professional

responsibility awareness and professional ethics in the development of professional identity (Travelbee, 1969).

This awareness enables nurses to act without deviating from ethical principles in patient care and to perform their profession in harmony with social values (Parola et al., 2020). Travelbee's approach aims to redefine the nursing profession as a profession based on humanitarian and ethical values, away from seeing it only as a technical task area (Shelton, 2016). In this context, it can be said that the human-to-human relationship, which is one of the basic elements of the model, plays a central role in the process of strengthening the professional identities of nurses (Brewington et al., 2023). In particular, the model, which explains the development of meaningful bonds established by the nurse with the patient in phases, allows us to better understand this process. The model explains the development of human to human relationship in five basic phases. These phases are; the original encounter, emerging identities, phase of empathy, phase of sympathy and phase of rapport (Travelbee, 1969).

2.1. Phase of the Original Encounter

This phase involves the first interaction between the nurse and the patient. Both parties observe each other and form first impressions based on verbal and non-verbal cues (Neff et al., 2024).

These impressions are often influenced by stereotypes and past experiences, as neither party knows the other personally at this point (Travelbee, 1969). First impressions or ‘first judgements’ are critical as they form the basis for future interactions (Neff et al., 2024). This phase is of great importance for nurses to display their professional stance and gain patient trust. These judgements may change as the relationship develops and more information is gathered.

As the initial interaction progresses, the relationship moves towards a more individualised understanding in the next phase (Travelbee, 1969).

2.2. Phase of Emerging Identities

As interactions progress, the unique identities of both the nurse and the patient gradually come into focus. This phase involves moving beyond preconceived stereotypes and recognising each other as distinct individuals (Travelbee, 1969). The emergence of identities plays a key role in helping nurses embrace their professional roles more transparently and consistently (Brewington & Godfrey, 2020). Replacing initial judgments with a deeper and more nuanced understanding, however, requires both time and continuous interaction (Travelbee, 1969).

This process is essential for breaking down preconceived notions and building a more personalised and effective caring relationship (Travelbee, 1969). As identities emerge and understanding deepens, the relationship can evolve further, supported by the development of empathy (Atta et al., 2024).

2.3. Phase of Empathy

According to Travelbee, empathy is a foundational element in the nurse-patient relationship. It entails understanding the patient's feelings and experiences from their perspective, allowing the nurse to develop a sense of emotional closeness to the patient (Atta et al., 2024). Travelbee emphasises that empathy is not

an immediate process; rather, it requires specific preconditions and a genuine willingness to deeply engage with the patient's situation (Travelbee, 1969).

The development of empathy enables nurses to adopt not only a patient-centered perspective but also emotional sensitivity and a human-centered approach as integral values of their professional identities (Müller et al., 2024). This process also helps nurses anticipate patient behaviours and tailor care to meet individual needs—an essential aspect of effective nursing practice (Mohan et al., 2025; Travelbee, 1969).

Empathy is thought to serve as a cornerstone in shaping professional identity, as it combines a sense of professional responsibility with deeply rooted human values. Building on this foundation, the relationship progresses into a phase of even deeper emotional connection: sympathy (Travelbee, 1969).

2.4. Phase of Sympathy

Sympathy represents a phase that transcends empathy by involving a shared emotional response to the patient's situation. This phase is characterised by a deeper connection, which can be achieved through deliberate cultivation and growth during the empathy phase.

The transition from empathy to sympathy not only enhances nurses' ability to form emotional connections but also ensures that the bond with the patient becomes more meaningful (Travelbee, 1969). In this context, sympathy is regarded as a powerful tool for strengthening the professional identity of nurses within the framework of the human-to-human relationship model. Developing sympathy allows nurses to deepen their sensitivity to the individual values and needs of the patient, fostering a therapeutic relationship that positively impacts patient outcomes (Younas, 2020).

As an advanced skill, sympathy can further enrich the nurse's patient-centred care approach, making significant contributions to the development of professional identity.

Sympathy, often intertwined with empathy, plays a key role in enhancing nurses' emotional awareness and in fostering meaningful relationships with patients (Pearson, 2021). At the same time, sympathy is thought to help nurses internalise their professional values, laying a stronger foundation for their professional identity. Once sympathy is established, the relationship reaches its most mature and harmonious state: rapport (Travelbee, 1969).

2.5. Phase of Rapport

According to Travelbee (1969), the main purpose of nurse-patient interaction is to develop a harmonious and friendly relationship based on mutual understanding, respect and co-operation. This relationship requires the nurse and the patient to work in partnership for the benefit of the patient (Travelbee, 1969).

This rapport provided in the relationship enable the nurse to perform her/his profession within the framework of human values and ethical principles by removing her/his professional identity from a task-oriented understanding (Koppel et al., 2022). It can be stated that these stages contribute significantly to the construction of professional nursing identity on the axis of human values as well as forming the basis of qualified care delivery. In

addition, the development of a harmonious nurse-patient relationship can expand the scope of professional identity by encouraging nurses to evaluate their profession not only as a field based on technical knowledge, but also with humanitarian and ethical dimensions (English et al., 2022).

Travelbee's human-to-human relationship model is thought to make a unique contribution to the development of professional identity by positioning empathy as an indispensable element of the nursing profession. The model's emphasis on understanding individual differences and exploring the depths of human behaviour expands the scope of nursing education while supporting professional development (Travelbee, 1969). Continuing professional education (Yu et al., 2022) and self-awareness studies provide a deeper understanding of nurses' roles and responsibilities and strengthen human values in patient care (Travelbee, 1969; Younas et al., 2020). Empathy enables the nurse not only to understand the patient but also to redefine their own human values and roles (Travelbee, 1969). Another important aspect of the model is its emphasis on balancing individual differences and shared experiences.

This balance enables the nurse to avoid overgeneralisations in patient care and to understand the unique needs of each individual (Mohan et al., 2025). For this purpose, Travelbee recommends the use of disciplines such as literature and art in the education process (Travelbee, 1969). Because these methods provide awareness of understanding individual differences and enable nurses to centre human values and individual needs in patient care (Schwind et al., 2014).

According to Travelbee, the main problem of nursing is the loss of the emotional bond with the patient (Travelbee, 1969). Today, time and resource constraints in the healthcare system further deepen this loss, leading to a task-oriented approach instead of patient-centred care (Kwame & Petrucka, 2021; Taleghani et al., 2018). This distancing weakens the emotional bonds that nurses establish with patients, increasing the risk of providing a robotic nursing service (Travelbee, 1969). This situation negatively affects patient satisfaction and professional satisfaction of nurses and reveals the lack of approaches that encourage establishing meaningful bonds with patients in nursing education (Lu et al., 2019). In the literature, it is stated that structured education programmes to improve nursing students‘interpersonal relationship and empathy skills are limited, which weakens students’ perception of professional identity (Beckstrom & Farrow, 2024; Oh, 2019; Peisachovich et al., 2024; Sung & Kweon, 2022). In this context, it is predicted that Travelbee's human-to-human relationship model can provide a theoretical basis to fill this gap.

3. Conclusion

In conclusion, Travelbee's human-to-human relationship model is a powerful tool that guides nurses to develop their professional identities and provide more compassionate, effective and holistic care. This model offers a perspective that reinforces the human-centred nature of the nursing profession and has the potential to improve the quality of health care at both the individual and societal levels. To fully realize the potential of Travelbee’s model, future research should explore its application in various educational and clinical settings. Investigating practical strategies to integrate this

framework into nursing curricula will not only enhance professional identity formation but also contribute to improved patient outcomes, greater job satisfaction among nurses, and the overall quality of healthcare.

By bridging the gap between technical expertise and emotional connection, Travelbee's model can play a pivotal role in shaping the future of compassionate and effective nursing care.

Ethical Statement

Ethics committee approval was deemed unnecessary for this study, given that open access sources were utilized.

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Presentation Information

The findings of this study have not been presented at any conference or journal.

Conflicts of Interest

The authors declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

Author Contributions

The contributions of the authors are as follows: İnci İnceleme contributed to literature search and editing the report. Satı Demir conducted the supervision and final revision of the manuscript.

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