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SYNTHESIS AND FUNCTIONAL PROPERTIES OF DIPHENYLAMINE-CONTAINING SCHIFF BASES

Hasan Ufuk CELEBIOGLU[1]*, Recep TAS[1], Ebru KOROGLU[1], Sevilay GÜNAY[2], Yavuz ERDEN[2]

[1]Department of Biotechnology,Faculty of Science,Bartin University,74100,Bartın
[2]Department of Molecular Biology and Genetics,Faculty of Science,Bartin University,74100,Bartın

Abstract

Schiff bases have been widely researched *in vitro* for their antimicrobial, antitumor, antiviral, antineoplastic, and antioxidant properties, owing to their strong chelating ability. Probiotics refer to "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Thus, this study aims to investigate the antibacterial, anticancer, and antioxidant effects of Schiff Bases *N*-methylene-*N*-phenylbenzenaminium and *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium, as well as their impact on some properties of probiotic bacteria, *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus*. Results indicate that these Schiff bases promote the growth of probiotic bacteria while inhibiting pathogenic bacteria. They demonstrate potent antibacterial and antioxidant activities, and cytotoxicity assays reveal that both compounds exert a high degree of inhibition on human colon and breast cancer cells. Additionally, there is significant modulation in auto-aggregation and surface hydrophobicity of *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus*. In conclusion, the Schiff bases investigated in this study possess significant therapeutic potential.

Key words: Antibacterial, anticancer, antioxidant, *Lactobacillus*, probiotics, schiff bases

1. Introduction

Schiff bases belong to a category of compounds, firstly determined by Hugo Schiff (Tidwell, 2008), that have garnered significant attention in recent years due to their diverse biological activities and produced These compounds are formed by the condensation of a primary amine with a carbonyl group, and they have a variety of applications in chemistry, biology, and pharmacology (Iftikhar et al., 2018; Liu et al., 2018). One of the most common applications of Schiff bases is in coordination chemistry, where they are frequently used as ligands due to their low cost and ease of synthesis (Abbo et al., 2005). Extensive *in vitro* researches have shown the significance of biological activities of Schiff bases. It is stated that the redox potentials of the central atom with the structure and conformation of the ligand affect the biological activities of such compounds (Durackova et al., 1999; Kumar et al., 2009). Due to their varying pharmacological properties, Schiff bases have gained considerable interest in the pharmacology, fields of chemistry and biology (Liberta & West, 1992, Kumar & Mishra, 2018). It is stated that Schiff bases have antimicrobial (Fioravanti et al., 1996; Khan et al., 2017), antitumor (Yang et al., 2000), antiviral (Das et al., 1999), antineoplastic (Sur et al., 1990), antitubercular (Krishna et al., 2014), anti-infective (Aly et al., 2020) properties. There are studies that show Schiff bases are good antioxidant compounds as they are excellent chelating agents (Galini et al., 2017). Additionally, they find applications as catalysts, organic synthesis intermediates, and stabilizers for paints, pigments, and polymers (Shanty et al., 2017). Furthermore, it is among the studies that Schiff bases show anticancer properties (Parekh et al., 2017).

On the other hand, probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" and known for their ability to improve intestinal balance and positively affect the host (Hill et al., 2014, Çelebi et al., 2020). To be a probiotic, microorganisms should bear some requirements. Firstly, they should have survival in the digestive system (Gonzalez-Rodriguez et al., 2013). The adhesion of probiotic bacteria to the gastrointestinal tract (GIT) is an important criterion for their effectiveness. This is because the mucus layer, covering the surface of the organs, is the first interacting site of microorganisms in the GIT, and the

***Sorumlu Yazar (Corresponding Author):**
Hasan Ufuk CELEBIOGLU; Bartın University, Faculty of Science, Department of Biotechnology 74100, Bartın-Turkey.

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adhesion of bacteria to this layer reduces pathogenic colonization and provides a healthy microbiota balance. The mucus layer is an important site for microbial adhesion and colonization as being the area that covers the surface of the organs and the first contact of microorganisms in the intestine. Because bacteria with aggregation ability form a barrier by binding to the surface and to each other (Collado et al., 2008). The adhesion properties of probiotic bacteria are determined by the composition and physical properties of their cell surface (Servin & Coconnier, 2003). Composition (proteins and lipids) on the bacterial surface and surface physical properties (e.g. surface hydrophobicity) of *lactobacilli* have generally roles in the adhesion properties of probiotic bacteria, yet these may differ among the strains (Buck et al., 2005; Percival et al., 2019). Thus, probiotic adhesion to mucus layer is important since it can reduce the pathogenic colonization, regulate immunological properties, and support healthy microbiota balance (Van Tassell & Miller, 2011). This may also play a role in the prevention of diseases related to the GIT (Siciliano & Mazzeo, 2012). For instance, a decreased amount of intestinal *lactobacilli* can be associated with ulcerative colitis, diarrhea, inflammatory bowel diseases, constipation, as well as colon cancer (Drisko et al., 2003).

Lactobacillus acidophilus and *Lacticaseibacillus rhamnosus* are *lactobacilli* that have been extensively studied. *L. acidophilus* has the ability to produce antimicrobial agents, which enable it to inhibit pathogenic bacteria. Studies have shown that *L. acidophilus* is present in the gastrointestinal tract more than any other microorganism, and it has been observed to inhibit different bacteria through the production of bacteriocin (Celebioglu & Svensson, 2018, Özçelik, 2014). *Lacticaseibacillus rhamnosus*, on the other hand, has been used as supplements and added to varying foods, including dairy products. This bacterium is capable of surviving in the acidic and alkaline conditions of the gastrointestinal tract, and has several potentials and uses in promoting digestive health (Capurso, 2019, Celebioglu et al., 2020a).

In the current study, the antibacterial, anticancer, and antioxidant activities of two diphenylamine-containing Schiff bases, *N*-methylene-*N*-phenylbenzenaminium and *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium, were evaluated. In addition, we evaluated the impact of these compounds on some probiotic properties of *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus*. Our study aims to contribute to the current understanding of the biological activities of Schiff bases and their potential therapeutic applications. Furthermore, our study sheds light on the potential effects of these compounds on probiotic bacteria, which can have promising applications.

2. Materials and Methods

2.1. Chemicals

All of chemicals (analytical grade) used in the Schiff base synthesis were purchased from Sigma-Aldrich and Fluka and used directly without further purification. The biological activities of the Schiff bases were measured at room temperature using (UV-Vis) spectrophotometer.

2.2. Synthesis of Schiff bases

The 250 mL sheathed flask was mixed with 10 mmol of formaldehyde and 5 mmol of diphenylamine solutions at room temperature, and reaction was continued by adding 75 mL anhydrous ethanol to the synthesis medium. The color of the medium changed from transparent to orange, and the resulting orange solution was refluxed continuously (36 h; 90°C; 400 rpm). Precipitate of Schiff base-1 (*N*-methylene-*N*-phenylbenzenaminium) was filtered with the help of Whatman No:1 filter paper, then washed with water, ethanol, and ether, respectively. The resulting Schiff base compounds were dried in a vacuum oven.

The ligands were synthesized by the condensation of 5 mmol glutaraldehyde and 5 mmol of diphenylamine in using ethanol (100 mL) as the reaction medium and were then, it was refluxed for 24 hours. After this it was put on cooling at room temperature and the solid products were obtained. The solvent in the obtained Schiff base was completely removed with the help of rotary evaporator. The precipitate of Schiff base-2 (*N*-(5-oxopentylidene)-*N*-phenylbenzenaminium) was filtered with the help of Whatman No:1 filter paper, then washed with water, ethanol, and ether, respectively. The resulting Schiff base compounds were dried in a vacuum oven under (Fig. 1).

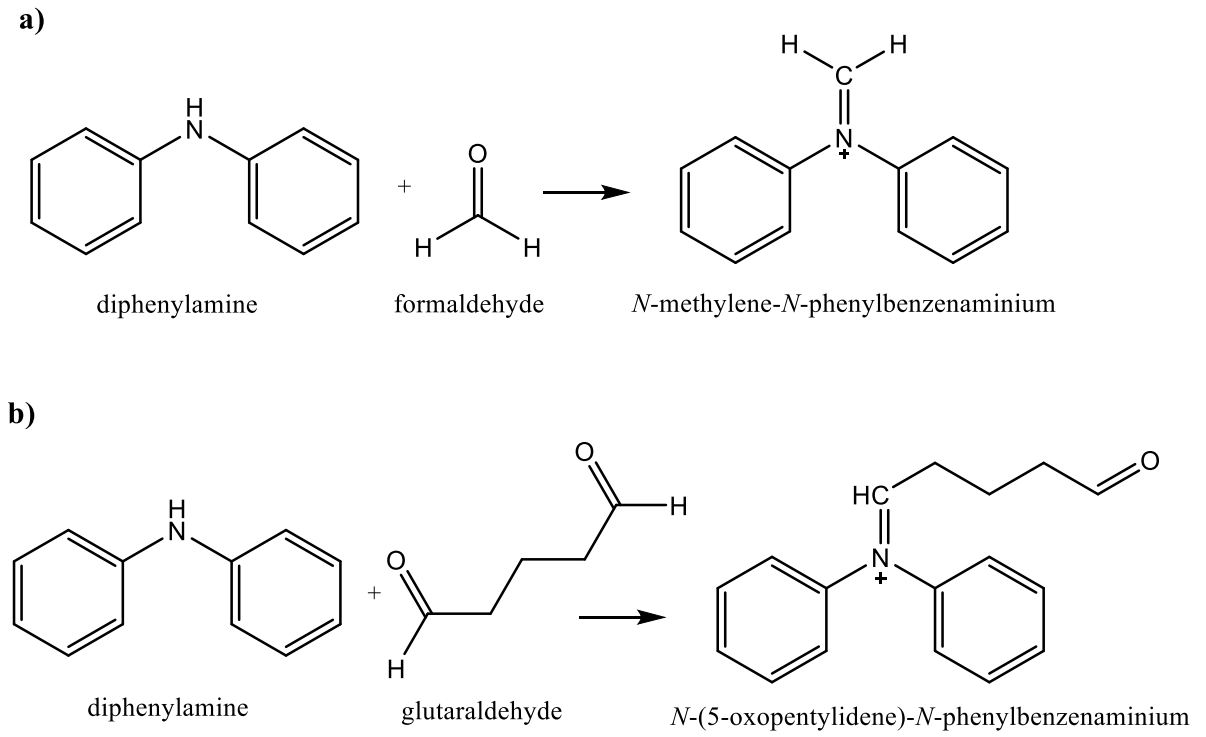

Fig.1. Reaction of Schiff Bases. (A) *N*-methylene-*N*-phenylbenzenaminium and (B) *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium

2.3. Antibacterial Activity Assay

To test the antibacterial properties of the Schiff bases, we employed Broth Micro-Dilution Assay, with some modifications based on the method of Brandt et al. (2010). We used *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), which were initially grown in Nutrient Broth (NB) for 24 hours at 37ºC. After sub-culturing, new cultures were grown until a density of 0.5 McFarland Unit reached. The bacterial cultures were then mixed with different concentrations of Schiff Bases (ranging from 0-1000 µg/mL) in microtiter plate wells containing NB. A negative control was included by using NB without bacteria. The microtiter plate was read at 0^{th} and 24^{th} hour at an absorbance of 600 nm in a micro-titer plate reader. Percentage inhibition versus compound concentration plot were plotted and the Minimum Inhibitory Concentrations (MICs) of the Schiff bases were calculated using these plots.

2.4. Anticancer Effect

2.4.1. Growth of cells

We examined the anticancer properties of Schiff bases for human colon (Caco-2 and HT-29) and breast (MCF-7) cancer cell lines. The cells were grown in DMEM (Caco2 and MCF-7) (Sigma-Aldrich, USA) or RPMI-1640 (HT-29) (Gibco, UK) medium supplemented with FBS (10%, Biowest, USA) and penicillin/streptomycin (1%, Gibco, UK) at 37°C with 5% CO2 (Thermo Forma II CO_2 incubator, USA).

2.4.2. Cell Viability (MTT assay)

Cytotoxic properties of the Schiff bases were evaluated using the MTT assay (Mosmann, 1983), where the cells were grown in 96-well microplates and treated with Schiff bases (1-100 µM) or DMSO as control for 24 hours. After incubation, MTT was added, followed by DMSO, and the absorbances were read at 570 nm (Thermo Multiskan Go, USA). The viability percentages of treated groups were calculated based on the absorbance values of the control group, which was considered 100% viable cells (Tekin et al., 2015).

2.5. DPPH Radical Scavenging Activity

DPPH stock solution was prepared before the experiment and covered with aluminum foil and transferred to the glass tube. 100, 500, 1000, 7500, 10000 µg/mL solutions of Schiff bases were prepared and 100 µL of these solutions were added with 100 µL DPPH to the 96-well plates. Only 96% ethanol and DPPH were added to the control groups. It was kept at RT for 30 minutes in the dark. Absorbance was read at 515 nm. The inhibition value of each mixture was calculated separately according to the equation (1).

$$\%\ DPPH\ Scavenging\ Activity = \left(1 - \frac{A_{Sample}}{A_{Control}}\right) \times 100 \quad (1)$$

2.6. Growth of Probiotics

Lactobacillus acidophilus LA-5 and *Lacticaseibacillus rhamnosus* GG (Chr. Hansen) were grown in a specific type of nutrient medium called Man, Rogosa and Sharpe (MRS) at 37ºC, without being shaken. Different concentrations of Schiff bases were added to the probiotics, while the control group was not treated with any Schiff bases. The determined concentration of Schiff base was added to the other groups. Bacterial growth was measured every four hours using a densitometer to investigate the effects of Schiff bases on the growth of the bacteria.

2.7. Surface Hydrophobicity (Microbial Adhesion to Solvents)

To determine the hydrophobicity of bacterial surfaces, the microbial adhesion to solvents (MATS) assay was used with some modifications, as described in previous studies by Celebioglu et al. (2016) and Köroğlu et al. (2019). The probiotics in the control and Schiff base-treatment groups were grown until stationary phase, followed by washing with PBS, and their OD600 was measured at 0.5 using a spectrophotometer. Next, they were suspended in 0.1 M KNO3 (pH 6.2) and mixed with xylene (a non-polar solvent) at a ratio of 3:1, followed by incubation at room temperature for 10 minutes. The tubes were then vortexed for 2 min, and the aqueous phase was separated and incubated for 20 minutes at room temperature. Absorbances were measured (600 nm) and microbial adhesions to solvent were calculated with the following equation (2).

$$\%\ Adhesion = \left(1 - \frac{A1}{A0}\right) \times 100 \quad (2)$$

where A1 represents the OD measurement after the specified period of incubation, while A0 represents the OD measurement prior to the start of incubation (Kos et al., 2003).

2.8. Auto-Aggregation

Probiotics were harvested before the stationary phase (3200xg, 15 min), washed with and re-suspended in PBS up to OD_{600} 0.5. Auto-aggregation was investigated by adding 4 mL dispersed bacteria to test tubes prepared for the experiment after vortexing for 10 seconds for one hour incubation at room temperature. After the incubation process, 100 µL of suspension was taken and added to the previously prepared tube containing 900 µL of PBS, and then mixed for a certain time. Then, absorbance measurement was performed at 600 nm. The percentage of auto-aggregation was calculated by the equation (3).

$$\%\ Autoaggregation = \left(1 - \frac{At}{A0}\right) \times 100 \quad (3)$$

Here At to represent the OD measured after a period of incubation, and A0 to represent the OD measured at the start of the experiment (0th hour) (Kos et al., 2003).

2.9. Statistical Analysis

Experiments were repeated at least thrice. The obtained results were analyzed with a statistical method called One-way Analysis of Variance (ANOVA) with additional pairwise comparisons (either Tukey's or Dunnett's Multiple Comparison Tests) using GraphPad Prism software version 5.0.0 for Windows, developed by GraphPad

Software in San Diego, California, USA (www.graphpad.com). The statistical significance level was set at $p<0.05$. Additionally, the software was used to calculate the $LogIC_{50}$ values to determine the toxic effects of the tested compounds on the cell lines.

3. Results

In this study, new Schiff bases were synthesized using the starting materials formaldehyde and diphenylamine for *N*-methylene-*N*-phenylbenzenaminium, and glutaraldehyde and diphenylamine for *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium.

3.1. Antibacterial Activity Assay

In this study, antibacterial effects of *N*-methylene-*N*-phenylbenzenaminium and *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium Schiff bases against *E. coli* and *S. aureus* were evaluated. Concentrations 0-1000 µg/mL of the Schiff bases were used in antibacterial activity assay, but the maximum concentration (1000 µg/mL) totally inhibited the bacterial growth in both strain. MIC values were calculated by drawing percentage inhibition vs. compound concentration plot.

For *E. coli*, MIC values of *N*-methylene-*N*-phenylbenzenaminium have been found as 252 µg/mL and for *S. aureus*, 269 µg/mL, and it has been observed that the bacterial viability rates of bacteria decreased proportionally with increasing concentration (Fig. 2). However, *N*-methylene-*N*-phenylbenzenaminium has less anti-bacterial impact on *S. aureus* than on *E. coli* (Fig. 2A). MIC values of *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium were calculated as 235 µg/mL for *E. coli*, and 207 µg/mL for *S. aureus* and bacterial viability rate decreased after 10 µg/mL with increasing concentration in *E.coli*, while bacterial viability rate decreased after 20 µg/mL with increasing concentration in *S. aureus*. (Fig. 2B). *N*-methylene-*N*-phenylbenzenaminium has been shown to have a higher antibacterial effect than *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium.

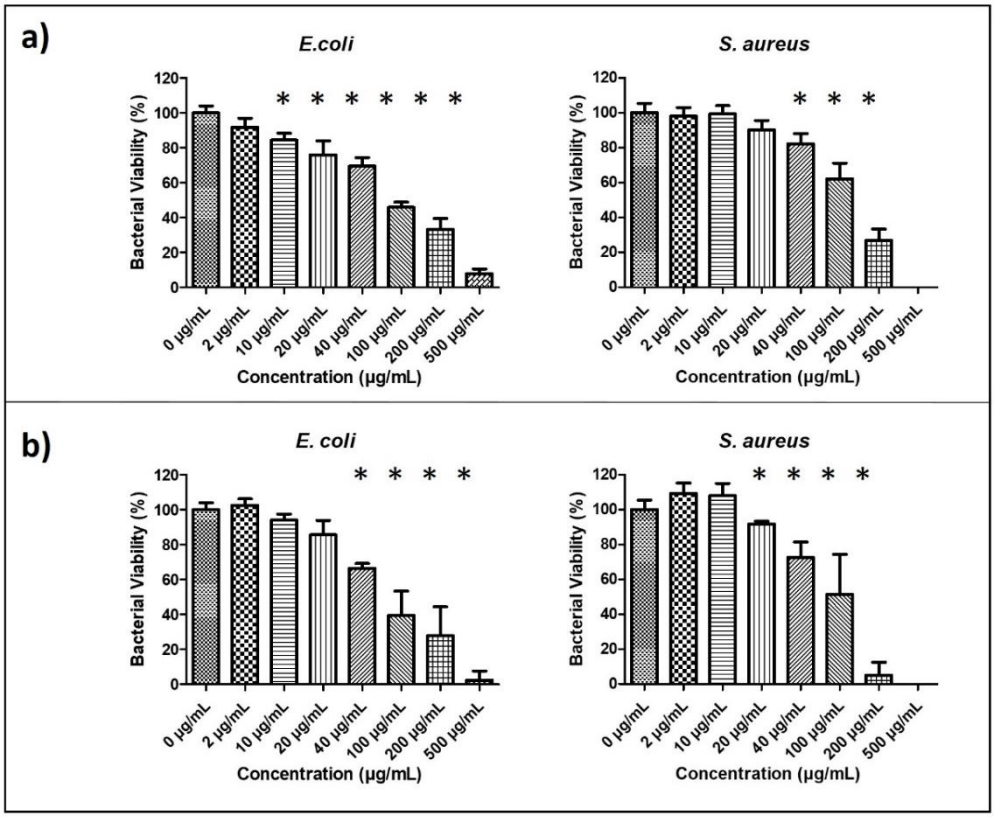

Fig. 2. Anti-bacterial Activities of Schiff bases. (A) *N*-methylene-*N*-phenylbenzenaminium and (B) *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium. * means statistically significant ($p<0.05$) when compared to control, using to One-Way ANOVA

3.2. Anticancer Effect

The change (%) determined in cell viability level after treatment with Schiff bases is shown in Figure 3 and Figure 4. The administered doses of 50 and 100 µM of *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium significantly reduced viability in all three cell lines compared to the control and vehicle groups (Figure 3; $p < 0.05$). The 25 µM dose of *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium showed a more pronounced effect in HT-29 and MCF-7 cells, and cell viability was significantly lower after this dose administration ($p < 0.05$). The other *N*-methylene-*N*-phenylbenzenaminium tested in the study was determined to cause cytotoxicity in cancer cells, similar to *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium. The dose of *N*-methylene-*N*-phenylbenzenaminium of 50 µM and above in Caco-2 and MCF-7, and doses of 25 µM and above in HT-29 cells significantly decreased the cell viability (Figure 4; $p < 0.05$). These results show us that both compounds exhibit similar cytotoxic effects in breast and colon cancer cells.

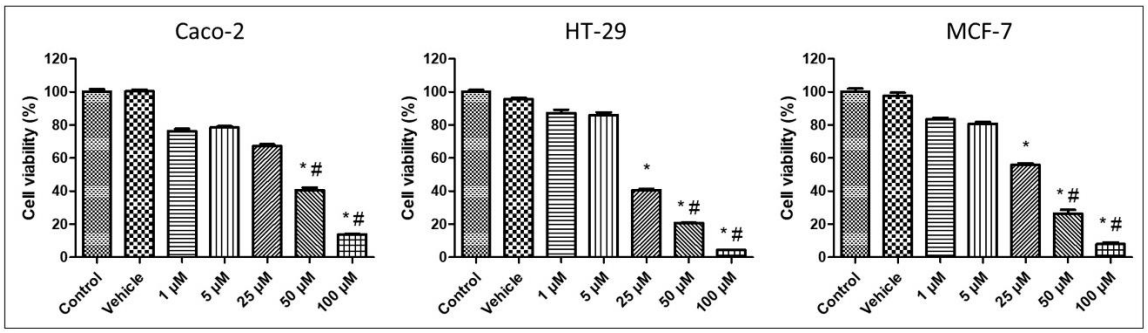

Fig. 3. Viability changes (%) in cell lines after *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium administration. Data are given as mean±S.D. (n = 5). * $p<0.05$ as compared to control; # $p<0.05$ as compared to vesicle control (DMSO).

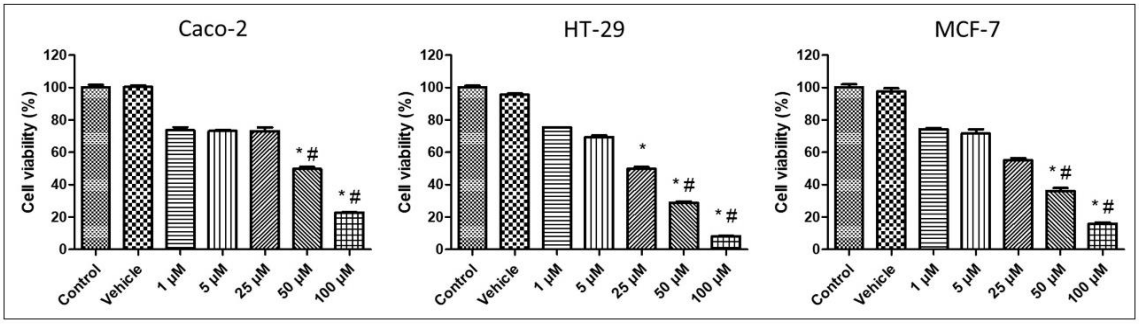

Fig. 4. Viability changes (%) in cell lines after *N*-methylene-*N*-phenylbenzenaminium administration. Data are given as mean±S.D (n = 5). * $p<0.05$ as compared to control; # $p<0.05$ as compared to vesicle control (DMSO).

$LogIC_{50}$ values as an indicator of the cytotoxic effects of Schiff bases on cell lines were calculated and presented in the Table 1.

Table 1. $LogIC_{50}$ values of test compounds (µM)

	***N*-(5-oxopentylidene)-*N*-phenylbenzenaminium**	***N*-methylene-*N*-phenylbenzenaminium**
Caco-2	1.44±0.21	1.58±0.29
HT-29	1.25±0.07	1.12±0.16
MCF-7	1.33±0.11	1.26±0.18

3.3. DPPH Radical Scavenging Activity

The radical scavenging activities of Schiff bases have been found to be increased with the increase in concentration (Fig. 5). DPPH scavenging effect starting from 13% in 50 µg/mL, up to 45% in 2500 µg/mL for *N*-methylene-*N*-phenylbenzenaminium, while 21% in 50 µg/mL, up to 63% in 5000 µg/mL for *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium.

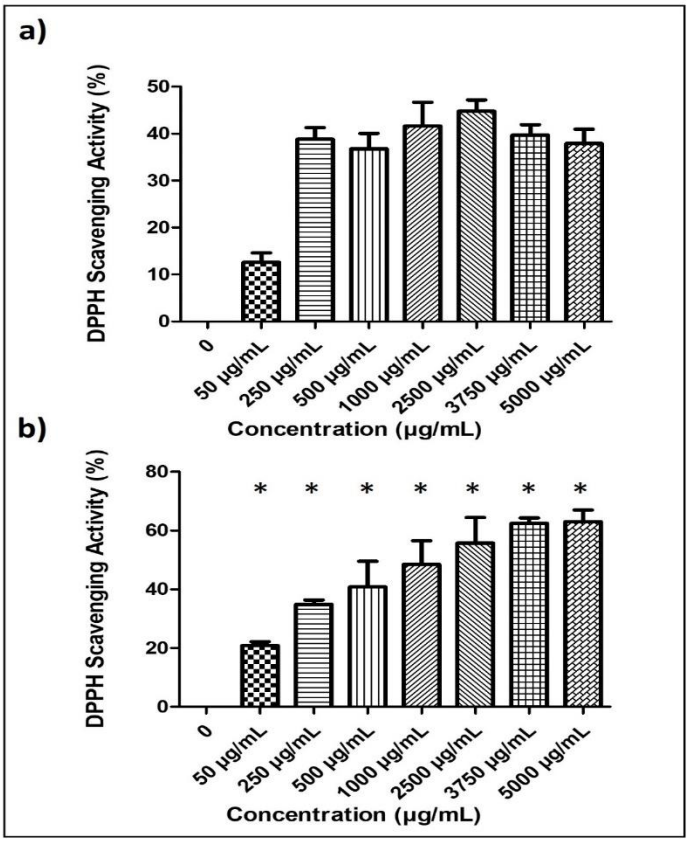

Fig. 5. Antioxidant Activities of Schiff bases. (A) *N*-methylene-*N*-phenylbenzenaminium and (B) *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium. * means $p<0.05$ when compared to control groups, using to One-Way ANOVA

3.4. Probiotic Properties of *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus*

Probiotics inoculated should pass a certain period of time until they adapt to the medium of growth and begin to multiply, and it is examined how much they grown within the specified period (Kedare & Singh, 2011; Mousavi et al., 2011).

In this study, none of the concentrations of Schiff bases had inhibitory effects on *L. acidophilus* and *L. rhamnosus* (Fig. 6), even at the MIC concentrations for *E. coli* and *S. aureus*. This means that these compounds selectively allow the growth of lactic acid bacteria, while inhibit the pathogenic bacteria like *E. coli* and *S. aureus*.

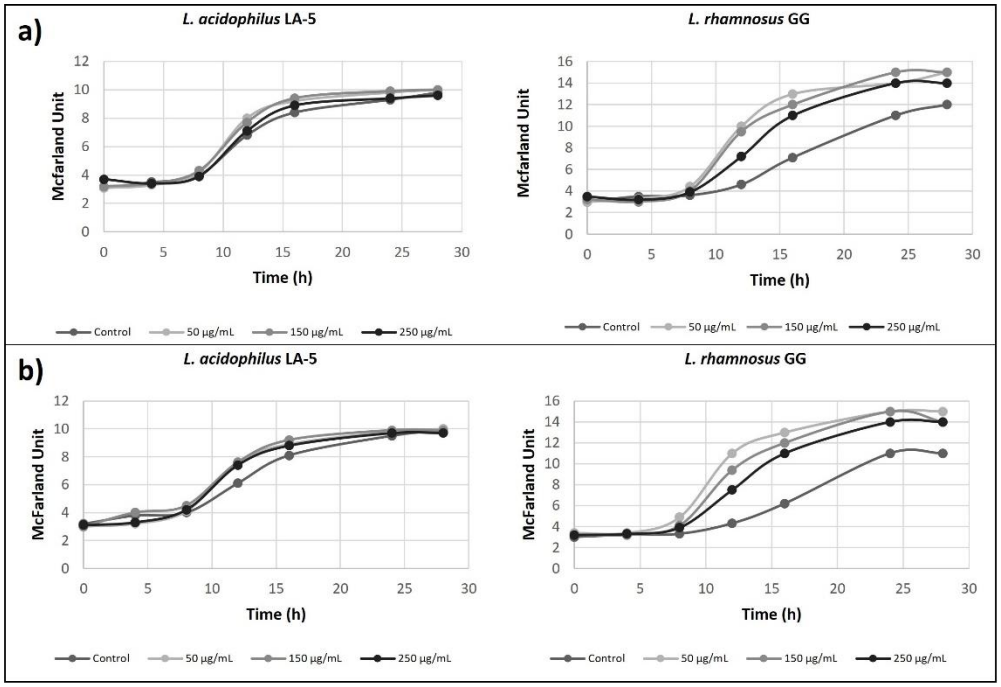

Fig. 6. Growth Curves of *L. acidophilus* LA-5 and *L. rhamnosus* GG treated with Schiff bases. (A) *N*-methylene-*N*-phenylbenzenaminium and (B) *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium

3.5. Surface Hydrophobicity

In our study, there was no general increase in surface hydrophobicity of probiotic bacteria (Fig. 7). However, *N*-methylene-*N*-phenylbenzenaminium increased the hydrophobicity of *L. acidophilus* LA-5 at the concentrations of 150 and 250 µg/mL (Fig. 7A). on the other hand, *N*- (5-oxopentylidene)-*N*-phenylbenzenaminium tends to decrease the surface hydrophobicity, but only concentration of 150 µg/mL significantly ($p<0.05$) decreased it (Fig. 7B).

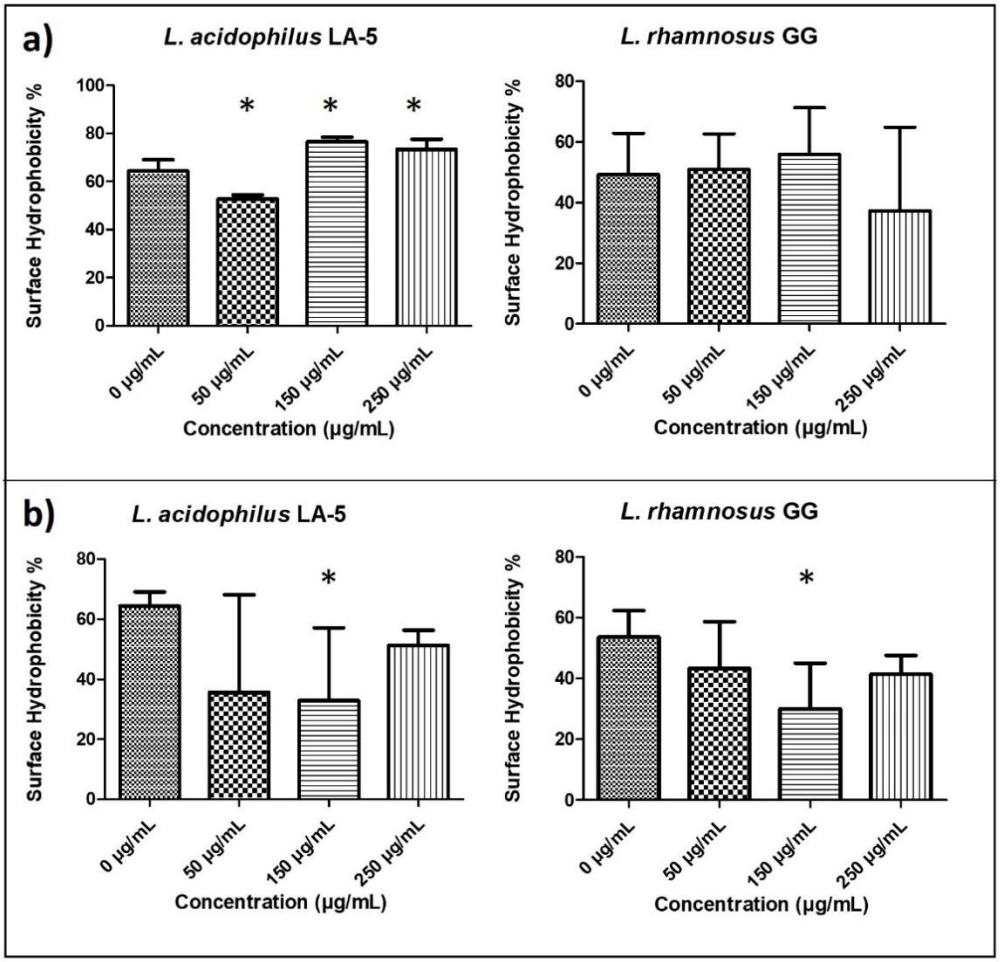

Fig. 7. Surface Hydrophobicity properties of *L. acidophilus* LA-5 and *L. rhamnosus* GG treated with Schiff bases, (A) *N*-methylene-*N*-phenylbenzenaminium and (B) *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium. * means $p<0.05$ when compared to control groups, using to One-Way ANOVA.

3.6. Auto-Aggregation

Adhesion of microorganisms to epithelial cells is associated with both hydrophobic property of the cell surface, as well as their aggregation capacities. Thus, in order for the probiotic bacteria to have a beneficial effect on the host, they should be sufficiently dense with their aggregation properties (Laparra & Sanz, 2009). It is an important property as when bacteria have high aggregation properties, this would allow them to colonize dominantly the GIT (Alander et al., 1999, Pedersen et al., 1989).

When looking at the auto-aggregation effects of *N*-methylene-*N*-phenylbenzenaminium and *N*-(5-oxopentylidene)-*N*-phenylbenzenaminium on probiotic bacteria, they both generally increased the auto-aggregation of *L. acidophilus* LA-5, while decreased that of *L. rhamnosus* GG (Table 2).

Table 2. Data on the auto-aggregation properties of probiotic bacteria after treatment with Schiff bases. Results are presented as the average value of three separate experiments, with standard deviations indicated in parentheses. The asterisks (*) indicate $p<0.05$ compared to control groups for each strain, as determined by the One-Way ANOVA statistical test.

***L. acidophilus* LA-5**	***1st***	***2nd***	***3rd***	***4th***	***5th*** ***Time (h)***
Control (0 µg/mL)	37.2 (11.0)	47.4 (14.2)	59.5 (11.7)	61.1 (0.2)	60.0 (5.0
N-methylene-N-phenylbenzenaminium (µg/mL)					
50	46.7 (25.3)	67.7 (12.6)*	89.6 (6.1)*	79.7 (3.9)*	94.8 (7.1)*
150	46.1 (25.3)	69.9 (12.3)*	86.9 (6.3)*	83.6 (9.7)*	98.8 (6.1)*
250	48.8 (29.0)	79.7 (10.8)*	78.2 (0.9)*	82.9 (10.9)*	95.6 (7.1)*
N-(5-oxopentylidene)-N-phenylbenzenaminium (µg/mL)					
50	38.4 (5.0)	54.6 (15.4)	70.9 (7.3)	77.2 (9.9)*	72.2 (11.0)*
150	54.7 (16.8)*	41.2 (3.4)	62.5 (10.9	79.3 (7.0)*	74.8 (2.3)*
250	26.2 (9.8)	19.1 (11.9)*	55.6 (23.7)	75.1 (13.5)*	79.4 (2.5)*
***L. rhamnosus* GG**	***1st***	***2nd***	***3rd***	***4th***	***5th*** ***Time (h)***
Control (0 µg/mL)	29.8 (2.2)	53.9 (9.2)	72.8 (9.1)	78.5 (9.2)	85.6 (5.5)
N-methylene-N-phenylbenzenaminium (µg/mL)					
50	21.7 (1.0)*	33.2 (8.8)*	36.5 (15.1)*	63.4 (6.7)*	70.0 (7.7)*
150	23.9 (6.3)*	34.1 (2.7)*	46.6 (13.8)*	52.8 (11.6)*	64.0 (4.8)*
250	28.3 (3.3)	40.5 (3.6)*	39.2 (5.8)*	53.4 (10.2)*	74.2 (14.6)*
N-(5-oxopentylidene)-N-phenylbenzenaminium (µg/mL)					
50	8.7 (6.9)*	6.8 (4.2)*	5.6 (3.0)*	5.6 (4.0)*	15.3 (3.5)*
150	7.0 (5.4)*	13.8 (3.6)*	14.6 (2.6)*	19.8 (4.1)*	27.5 (1.8)*
250	11.7 (3.5)*	6.9 (2.1)*	10.1 (8.3)*	19.3 (5.7)*	15.2 (4.8)*

4. Discussion

The study on Schiff bases is an area that is being recognized. They are prepared by the reaction of an aldehyde and primary amines. The C=N bond in Schiff bases is a structural requirement for biological activity. For this reason, Schiff bases have been shown to exhibit varying biological activities such as antibacterial, antioxidant, and antifungal properties (Iqbal et al., 2007). There are azometin and imine groups in various natural and unnatural compounds. The imine group found in such compounds has been shown to be effective in biological activities (Bringmann et al., 2004). Because of these properties, Schiff bases are considered as a promising antibacterial agent (Taslimi et al., 2021). For example, the antimicrobial effect of the Schiff base obtained from primary amine and 5-chloro-salicylaldehyde has recently been reported (De Souza et al., 2007). The Schiff bases evaluated were found to be effective on at least one bacterial strain at 2.5 - 5.2 µg/mL MIC values (De Souza et al., 2007).

DPPH, a stable free radical, takes up a hydrogen or electron radical. The lower the absorbance values read at 515 nm in DPPH, the higher the free radical removal activity (Shi et al., 2007). The removal of free radical after the exchange of hydrogen between antioxidant and free radical causes the absorbance to decrease (Shi et al., 2007). Schiff bases act as antioxidants thanks to the hydroxyl groups they contain in their chemical structure and can protect the harmful effects of free radicals (Mamedova et al., 2019) . Their antioxidant capabilities were evaluated using the DPPH method, which involves measuring the ability of compounds to transfer electrons to reactive radicals, thus neutralizing them. According to the results, we found that both display excellent antioxidant possessed good radical scavenging effects for DPPH radicals.

Cancer, one of the leading health problems of today, represents a heterogeneous group of diseases that develop as a result of gene mutations in cells and consequent dysfunction in metabolic processes. This heterogeneity and the different physiological and pharmacological responses of the treated individuals are the most important factors in reducing the success rate of cancer treatment (Dagogo-Jack & Shaw, 2018). The undesirable side effects of drugs used in cancer treatment on healthy cells also make this process even more complex. Numerous studies to improve this condition and increase treatment success are targeting the identification of potential therapeutics or the synthesis of new compounds that can be used in cancer treatment. Numerous studies show that Schiff bases exhibit strong cytotoxicity on cancer cells. Abd-Elzaher et al. investigated the cytotoxic effects of the new Schiff base compounds against breast, liver, lung, and colorectal cancer cell lines. The researchers reported that the ligand they synthesized exerted an effect on three other cancer cells besides the A549 cell; moreover, the ZnII complex derived from this ligand caused strong cytotoxicity in all cell lines (Abd-Elzaher et al., 2016). Another study reports that vanillin Schiff base-derived copper (II) complexes exhibit high nuclease activity, killing cancer cells, including cancer stem cell enriched cells. Furthermore, some compounds synthesized in the same study were shown to be easily taken up by cancer stem cells, increase the levels of intracellular reactive oxygen species, cause DNA damage, as well induce caspase-dependent apoptosis (Lu et al., 2017). Many other studies report that Schiff base molecules have a strong cytotoxic effect against cancer cells and that these compounds may be potential drug candidates (Celebioglu et al., 2020b; Hajrezaie et al., 2014; Xia et al., 2019). Here, the cytotoxic properties of the synthesized compounds on breast (MCF-7) and colon cancer cell lines (HT-29 and Caco-2) were investigated. Our results showed that our test compounds exhibited a significant cytotoxic effect on all three cancer cells. In particular, the application of N-(5-oxopentylidene)-N-phenylbenzenaminium caused a more pronounced decrease in cell viability. These results indicate that Schiff base compounds can be effective in cancer treatment as stated in the literature.

Complementary and alternative medicine increases its importance day by day and reveals the importance of herbs taken with diet. *In vivo* and *ex vivo* studies have been conducted to investigate the effects of dietary compounds on intestinal microbiota. Evaluating the effect of food components on the characteristic microorganisms of microbiota can be useful in obtaining important information (Charalampopoulos et al., 2002). In a study by Sutherland et al. (2009), they showed that using food extracts has direct prebiotic and antibacterial effects on intestinal bacteria. In particular they showed that garlic and black pepper significantly increase the growth of *L. reuteri*, a probiotic bacterium, and inhibit *E. coli* strains, which are pathogenic bacteria (Michel-Barba et al.,2019). Garlic has antibacterial properties against *E. coli*, *S. aureus*, *P. aeruginosa* pathogens (Bayani & Azanza, 2005; Bjarnsholt et al., 2005; Sutherland et al., 2009). This activity is an important feature in increasing the growth of probiotic bacteria. Probiotics are useful for microbiota, and these bacteria are best known for their ability to stimulate host resistance to pathogens (Gupta & Ravishankar, 2005). For example, *Lactobacillus* has a significant effect on microbiota, because they have the ability to lower pH in the microbiota environment and produce short chain fatty acids to reduce pathogens (Roy, 1992). Furthermore, probiotic bacteria are generally resistant to especially host digestive systems (like gastrointestinal enzymes) and also are stronger than other bacteria (Holmes et al., 2012). This can be the explanation why they dominate the

gastrointestinal tract in the body and they compete and fight with the pathogenic bacteria. Thus, it can be reasonable that such probiotic bacteria are hardly affected by agents but not pathogenic bacteria. This is also a favorable condition for an ingredient or potential agent that does not affect probiotic bacteria, but inhibiting the pathogens.

Intestines have mucosal surface, which is an important place for bacterial colonization and adhesion (Collado et al., 2008). In order for probiotic bacteria not to disappear from the surface of the GIT, it is necessary to attach to the epithelial cells and the layer of mucus that covers the intestinal lumen. With this attachment, the probability of probiotics remaining in the digestive system is a longer, preventing the attachment of pathogens to the intestinal surface, activating the immune system and healing the damaged intestinal epithelium more easily (Holmes et al., 2012). Thus, adhesion of beneficial microorganisms to the mucus layer of GIT is of paramount importance and this adhesion is related with some properties of the bacterial surfaces (Celebioglu & Svensson, 2018). One of these properties is bacterial surface hydrophobicity, the higher the surface hydrophobicity, the more likely the beneficial bacteria will bind to the intestinal mucosa (Arena et al., 2017).

5. Conclusions

In this research, effects of Schiff bases on the bacterial growth kinetics, bacterial surface hydrophobicity, bacterial auto-aggregation properties of *L. acidophilus* and *L. rhamnosus*, as well as anti-microbial effects on pathogenic bacteria (*E. coli* and *S. aureus*) were investigated. Obtained Schiff bases had strong cytotoxic effects on human breast and colon cancer cell lines. We also explained that Schiff bases allow the development of probiotic bacteria, while inhibiting pathogenic bacteria. They also altered the probiotic properties of *lactobacillus* strains. Even though the Schiff bases investigated in the present study are potential therapeutics, comprehensive studies can be conducted such as the effects of these compounds on gene and protein expressions. Furthermore, by evaluating these properties, it can be a horizon for further studies in this field.

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References

1. **Abbo, H.S., Titinchi, S.J.J., Prasad, R. & Chand, S. (2005).** Synthesis, characterization and study of polymeric iron (III) complexes with bidentate p-hydroxy Schiff bases as heterogeneous catalysts. *Journal of Molecular Catalysis a-Chemical*.;225(2), 225-232.
2. **Abd-Elzaher, M.M., Labib, A.A., Mousa, H.A., Moustafa, S.A., Ali, M.M. & El-Rashedy, A.A. (2016).** Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety. *Beni-Suef University Journal of Basic and Applied Sciences*.5(1), 85-96.
3. **Alander, M., Satokari, R., Korpela, R., Saxelin, M., Vilpponen-Salmela, T. Mattila-Sandholm, T. & von Wright, A. (1999).** Persistence of colonization of human colonic mucosa by a probiotic strain, Lactobacillus rhamnosus GG, after oral consumption. *Appl. Environ. Microbiol.*, *65*(1), 351-354.
4. **Aly, A.A., Hassan, A.A., Makhlouf, M.M. & Bräse, S. (2020).** Chemistry and biological activities of 1,2,4-triazolethiones—antiviral and anti-infective drugs. *Molecules.* 25(13), 30-36.
5. **Arena, M.P., Capozzi, V., Spano, G. & Fiocco, D. (2017).** The potential of lactic acid bacteria to colonize biotic and abiotic surfaces and the investigation of their interactions and mechanisms. *Applied Microbiology and Biotechnology*, 101(7), 2641-2657.
6. **Bayani, M.A. & Azanza, M.P.V. (2005**). Inhibition of Staphylococcus aureus by garlic and NaCl in broth systems. *Food Science and Technology Research*.11(2), 214-221.
7. **Bjarnsholt, T., Jensen, P.O., Rasmussen, T.B., Christophersen, L., Calum, H., Hentzer, M., Hougen, H.P., Rygaard, J., Moser, C., Ebert, L., Hoiby, N. & Givskov, M. (2005).** Garlic blocks quorum sensing and promotes rapid clearing of pulmonary Pseudomonas aeruginosa infections. *Microbiology-Sgm.* 151, 3873-3880.

8. **Brandt, A.L., Castillo, A., Harris, K.B., Keeton, J.T., Hardin, M.D. & Taylor, T.M. (2010).** Inhibition of Listeria monocytogenes by Food Antimicrobials Applied Singly and in Combination. *Journal of Food Science*. 75(9), M557-M563.

9. **Bringmann, G., Dreyer, M., Faber, J.H., Dalsgaard, P.W., Staerk, D., Jaroszewski, J.W., Ndangalasi, H., Mbago, F., Brun, R. & Christensen, S.B. (2004).** Ancistrotanzanine C and related 5,1 '- and 7,3 '-coupled naphthylisoquinoline alkaloids from Ancistrocladus tanzaniensis. *Journal of Natural Products*. 67(5), 743-748.

10. **Buck, B.L., Altermann, E., Svingerud, T. & Klaenhammer, T.R. (2005).** Functional analysis of putative adhesion factors in Lactobacillus acidophilus NCFM. *Applied and Environmental Microbiology*. 71(12), 8344-8351.

11. **Capurso, L. (2019).** Thirty years of *Lactobacillus rhamnosus* GG A Review. *Journal of Clinical Gastroenterology*. 53, S1-S41.

12. **Çelebi, B., Taş, R., Akşit, A. & Celebioglu, H.U. (2020).** Effects of loganic acid isolated from *Vinca soneri* on surface hydrophobicity and auto-aggregation of probiotic bacteria, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *Erzincan University Journal of Science and Technology*. 13(1), 115-122.

13. **Celebioglu, H.U., Ejby, M., Majumder, A., Kobler, C., Goh, Y.J., Thorsen, K., Schmidt, B., Flaherty, S., Hachem, M.A., Lahtinen, S.J., Jacobsen, S., Klaenhammer, T.R., Brix, S., Molhave, K. & Svensson, B. (2016).** Differential proteome and cellular adhesion analyses of the probiotic bacterium *Lactobacillus acidophilus* NCFM grown on raffinose - an emerging prebiotic. *Proteomics*. 16(9), 1361-1375.

14. **Celebioglu, H.U., Kesici, A. & Taş, R. (2020a)**. Investigation of possibilities of using *Nerium oleander* L. extract as prebiotic for *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *Erzincan University Journal of Science and Technology*. 13(3), 1147-1157.

15. **Celebioglu, H.U., Erden, Y., Hamurcu, F., Taslimi, P., Şentürk, O.S., Özmen, Ü.Ö., Tuzun, B. & Gülçin, İ. (2020b).** Cytotoxic effects, carbonic anhydrase isoenzymes, α-glycosidase and acetylcholinesterase inhibitory properties, and molecular docking studies of heteroatom-containing sulfonyl hydrazone derivatives. *Journal of Biomolecular Structure and Dynamics*, 1-12.

16. **Celebioglu, H.U. & Svensson, B. (2018).** Dietary nutrients, proteomes, and adhesion of probiotic lactobacilli to mucin and host epithelial cells. *Microorganisms*. 6(3).

17. **Charalampopoulos, D., Pandiella, S.S. & Webb, C. (2002).** Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. *Journal of Applied Microbiology*. 92(5), 851-859.

18. **Collado, M.C., Meriluoto, J. & Salminen, S. (2008).** Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology*. 226(5), 1065-1073.

19. **Dagogo-Jack, I & Shaw, A.T. (2018).** Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol.* 15(2), 81-94.

20. **Das, A., Trousdale, M.D., Ren, S.J. & Lien, E.J. (1999).** Inhibition of herpes simplex virus type 1 and adenovirus type 5 by heterocyclic Schiff bases of aminohydroxyguanidine tosylate. *Antiviral Research.* 44(3), 201-208.

21. **De Souza, A.O., Galetti, F.C.S., Silva, C.L., Bicalho, B., Parma, M.M., Fonseca, S.F., Marsaioli, A.J., Trindade, A.C., Gil, R.P., Bezerra, F.S., Neto, M.A. & Oliveira, M.C. (2007).** Antimycobacterial and cytotoxicity activity of synthetic and natural compounds. *Quimica Nova.* 30(7), 1563-1566.

22. **Drisko, J.A., Giles, C.K. & Bischoff, B.J. (2003).** Probiotics in health maintenance and disease prevention. *Alternative medicine review*.8(2), 143-155.

23. **Durackova, Z., Mendiola, M.A., Sevilla, M.T. & Valent, A. (1999).** Thiohydrazone copper(II) complexes. The relationship between redox properties and superoxide dismutase mimetic activity. *Bioelectrochemistry and Bioenergetics.* 48(1), 109-116.

24. **Fioravanti, R., Biava, M., Donnarumma, S. & Porretta, G.C. (1996).** Synthesis and microbiological evaluations of (N-heteroaryl)arylmethanamines and their Schiff bases. *Farmaco.* 51(10), 643-652.

25. **Galini, M., Salehi, M., Kubicki, M., Amiri, A. & Khaleghian, A. (2017).** Structural characterization and electrochemical studies of Co(II), Zn(II), Ni(II) and Cu(II) Schiff base complexes derived from 2-((E)-(2-

methoxyphenylimino)methyl)-4-bromophenol; Evaluation of antioxidant and antibacterial properties. *Inorganica Chimica Acta.* 461, 167-173.

26. **Gonzalez-Rodriguez, I., Ruiz, L., Gueimonde, M., Margolles, A. & Sanchez, B. (2013).** Factors involved in the colonization and survival of bifidobacteria in the gastrointestinal tract. *Fems Microbiology Letters*. 340(1), 1-10.

27. **Gupta, S. & Ravishankar, S. (2005).** A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against Escherichia coli O157 : H7 in laboratory buffer and ground beef. *Foodborne Pathogens and Disease*. 2(4), 330-340.

28. **Hajrezaie, M., Paydar, M., Zorofchian Moghadamtousi, S., Hassandarvish, P., Gwaram, N.S., Zahedifard, M., Rouhollahi, E., Karimian, H., Looi, C.Y., Ali, H.M., Majid, N.A. & Abdulla, M.A. (2014).** A Schiff Base-derived copper (II) complex is a potent inducer of apoptosis in colon cancer cells by activating the intrinsic pathway. *The Scientific World Journal.*

29. **Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C. & Sanders, M.E. (2014).** The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology.* 11(8), 506-514.

30. **Holmes, E., Li, J.V., Marchesi, J.R.. & Nicholson, J.K. (2012).** Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metabolism.* 16(5), 559-564.

31. **Iftikhar, B., Javed, K., Khan, M.S.U., Akhter, Z., Mirza, B. & Mckee, V. (2018).** Synthesis, characterization and biological assay of Salicylaldehyde Schiff base Cu(II) complexes and their precursors. *Journal of Molecular Structure*. 1155, 337-348.

32. **Iqbal, A., Siddiqui, H.L., Ashraf, C.M., Ahmad, M. & Weaver, G.W. (2007).** Synthesis, characterization and antibacterial activity of azomethine derivatives derived from 2-formylphenoxyacetic acid. *Molecules*. 12(2), 245-254.

33. **Kedare, S.B. & Singh, R.P. (2011).** Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology-Mysore*. 48(4), 412-422.

34. **Khan, S.A., Asiri, A.M. & Sharma, K. (2017).** Efficient microwave assisted synthesis and computational study of isoxazole Schiff base as an antibacterial agent. *Indian Journal of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry*. 56(4), 453-457.

35. **Krishna, K.M., Inturi, B., Pujar, G.V., Madhusudan, N.P. & Vijaykumar, G.S. (2014).** Design, synthesis and 3D-QSAR studies of new diphenylamine containing 1,2,4-triazoles as potential antitubercular agents. *European Journal of Medicinal Chemistry*, 84(12), 516-529.

36. **Kos, B., Suskovic, J., Vukovic, S., Simpraga, M., Frece, J. & Matosic, S. (2003).** Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology.* 94(6), 981-987.

37. **Köroğlu, E., Celebioglu, H.U., Akşit, H. & Taş, R. (2019).** Insight into effects of ipolamiide isolated from *Plantago euphratica* on probiotic properties of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *European Journal of Science and Technology*. 17, 995-1000.

38. **Kumar, S., Dhar, D.N. & Saxena, P.N. (2009).** Applications of metal complexes of Schiff bases-A review. *Journal of Scientific & Industrial Research.* 68(3), 181-187.

39. **Kumar, A. & Mishra, A.K. (2018).** Pharmacological applications of diphenylamine and its derivative as potent bioactive compound: A Review. *Current Bioactive Compounds.* 14(3), 217-233.

40. **Laparra, J.M. & Sanz, Y. (2009).** Comparison of in vitro models to study bacterial adhesion to the intestinal epithelium. *Letters in Applied Microbiology.* 49(6), 695-701.

41. **Liberta, A.E. & West, D.X. (1992).** Antifungal and antitumor-activity of heterocyclic thiosemicarbazones and their metal-complexes - Current Status. *Biometals.* 5(2), 121-126.

42. **Liu, Y.T., Sheng, J., Yin, D.W., Xin, H., Yang, X.M., Qiao, Q.Y. & Yang, Z.J. (2018).** Ferrocenyl chalcone-based Schiff bases and their metal complexes: Highly efficient, solvent-free synthesis, characterization, biological research. *Journal of Organometallic Chemistry.* 856, 27-33.

43. **Lu, C., Eskandari, A., Cressey, P.B. & Suntharalingam, K. (2017).** Cancer stem cell and bulk cancer cell active Copper(ii) complexes with vanillin Schiff base derivatives and naproxen. *Chemistry – A European Journal.* 23(47), 11366-11374.

44. **Mamedova, G., Mahmudova, A., Mamedov, S., Erden, Y., Taslimi, P., Tüzün, B., Tas, R., Farzaliyev, V., Sujayev, A., Alwasel, S.H. & Gulçin, İ. (2019).** Novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines: Synthesis, characterization, antidiabetic, anticancer, anticholinergic, and molecular docking studies. Bioorganic Chemistry 93, 103313.

45. **Michel-Barba, M.G., Espinosa-Andrews, H., Garcia-Reyes, R.A., Desjardins, Y. & Gonzalez-Avila, M. (2019).** Effect of blueberry extract, carriers, and combinations on the growth rate of probiotic and pathogenic bacteria. *International Journal of Food Sciences and Nutrition.* 70(1), 63-70.

46. **Mosmann, T. (1983).** Rapid colorimetric assay for cellular growth and survival - application to proliferation and cyto-toxicity assays. *Journal of Immunological Methods.* 65(1-2), 55-63.

47. **Mousavi, Z.E., Mousavi, S.M., Razavi, S.H., Emam-Djomeh, Z. & Kiani, H. (2011).** Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of Microbiology & Biotechnology.* 27(1), 123-128.

48. **Özçelik, G.G.F. (2014).** Probiotic use of bacteriocin producing lactic acid bacteria. *Academic Food.* 12(1), 63-68.

49. **Parekh, N.M., Mistry, B.M., Pandurangan, M., Shinde, S.K. & Patel, R.V. (2017).** Investigation of anticancer potencies of newly generated Schiff base imidazolylphenylheterocyclic-2-ylmethylenethiazole-2-amines. *Chinese Chemical Letters.* 28(3), 602-606.

50. **Pedersen, K. & Tannock, G.W. (1989).** Colonization of the porcine gastrointestinal tract by lactobacilli. *Appl. Environ. Microbiol., 55*(2), 279-283.

51. **Percival, G.C., Chamundeeswari, M., Lovlyna, F.R., Seethalakshmi, R. & Sreekumar, G. (2019).** Production and partial purification of beta-galactosidase enzyme from probiotic Bacillus subtilis SK09. *Indian Journal of Biotechnology.* 18(2), 139-144.

52. **Roy, F. (1992).** History and development of probiotics. In: Probiotics. In. Dordrecht: Springer.

53. **Servin, A.L. & Coconnier, M.H. (2003).** Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. *Best Practice & Research Clinical Gastroenterology.* 17(5), 741-754.

54. **Shanty, A.A., Philip, J.E., Sneha, E.J., Kurup, M.R.P., Balachandran, S. & Mohanan, P.V. (2017).** Synthesis, characterization and biological studies of Schiff bases derived from heterocyclic moiety. *Bioorganic Chemistry.* 70, 67-73.

55. **Shi, L., Ge, H.M., Tan, S.H., Li, H.Q., Song, Y.C., Zhu, H.L. & Tan, R.X. (2007).** Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. *European Journal of Medicinal Chemistry.* 42(4), 558-564.

56. **Siciliano, R.A. & Mazzeo, M.F. (2012).** Molecular mechanisms of probiotic action: a proteomic perspective. *Current Opinion in Microbiology.* 15(3), 390-396.

57. **Sur, B., Chatterjee, S.P., Sur, P., Maity, T. & Roychoudhury, S. (1990).** Studies on the Antineoplasticity of Schiff-Bases Containing 5-Nitrofuran and Pyrimidine. *Oncology*. 47(5), 433-438.

58. **Sutherland, J., Miles, M., Hedderley, D., Li, J., Devoy, S., Sutton, K. & Lauren, D. (2009).** In vitro effects of food extracts on selected probiotic and pathogenic bacteria. *International Journal of Food Sciences and Nutrition.* 60(8), 717-727.

59. **Taslimi, P., Erden, Y., Mamedov, S., Zeynalova, L., Ladokhina, N., Tas, R., Tuzun, B., Sujayev, A., Sadeghian, N., Alwasel, S.H. & Gulcin, İ. (2021).** The biological activities, molecular docking studies, and anticancer effects of 1-arylsuphonylpyrazole derivatives. *Journal of Biomolecular Structure and Dynamics* 39(9), 3336-3346.

60. **Tekin, S., Erden, Y., Sandal, S. & Yilmaz, B. (2015).** Is irisin an anticarcinogenic peptide? *Medicine Science.* 4(2), 2172-2180.

61. **Tidwell, T.T. (2008).** Hugo (ugo) Schiff, Schiff bases, and a century of beta-lactam synthesis. *Angewandte Chemie-International Edition.* 47(6), 1016-1020.

62. **Van Tassell, M.L. & Miller, M.J. (2011).** *Lactobacillus* Adhesion to Mucus. *Nutrients*. 3(5), 613-636.

63. **Xia, Y., Liu, X., Zhang, L., Zhang, J., Li, C., Zhang, N., Xu, H. & Li, Y. (2019).** A new Schiff base coordinated copper(II) compound induces apoptosis and inhibits tumor growth in gastric cancer. *Cancer Cell Int.* 19, 81.

64. **Yang, Z.Y., Yang, R.D., Li, F.S. & Yu, K.B. (2000).** Crystal structure and antitumor activity of some rare earth metal complexes with Schiff base. *Polyhedron*. 19(26-27), 2599-2604.