**AI Verification for Spirulina's Antimicrobial Power in Total Coliform and *Staphylococcus aureus* Isolated from Tilapia Fillet**

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**Abstract**Seafood products, including fresh tilapia fillets, are highly susceptible to rapid quality deterioration due to microbial contamination, posing a significant concern for food safety and public health. This study investigated, both experimentally and through artificial intelligence modeling, the antimicrobial activity of Spirulina platensis (SP) extracts against two common foodborne pathogens: total coliforms and Staphylococcus aureus (S. aureus). SP extracts were obtained using the freeze-thaw method at concentrations of 0.5%, 1%, and 5% (w/v), labeled as EA, EB, and EC, respectively. Microbial analysis was conducted on 25 fresh Nile tilapia fillets. Each fish was divided into four quarters: one served as the control, while the remaining three were treated with the different SP extract concentrations. Bacterial counts were recorded before treatment and at 1, 24, and 48 hours post-treatment, with samples stored at 4°C throughout. The antimicrobial activity of the SP extracts was modeled using Artificial Neural Network (ANN) and Adaptive Neuro-Fuzzy Inference System (ANFIS) models. The results indicated that SP extracts effectively reduced total coliform and S. aureus counts in the treated fish fillets. Both ANN and ANFIS models accurately predicted the reduction in microbial counts, validating the experimental results. Among the treatments, EC (5% concentration) exhibited the highest antimicrobial activity, outperforming EB and EA. These findings suggest that SP extracts hold promise as a natural preservative for seafood and other food products, contributing to food safety and public health protection. Future research should focus on identifying and characterizing the bioactive compounds in SP extracts across different food matrices, with potential applications in the development of functional foods and novel therapeutics.

Keywords: *Spirulina* *platensis* extracts, antimicrobial activity, fish fillets, Total coliform,

*S. aureus*, artificial intelligence.

**Introduction**

Fish and seafood are widely regarded as high-quality food products that offer health benefits and hold substantial commercial value. However, their high perishability results in a short shelf life [1, 2]. The deterioration of fishery products begins immediately after they are removed from their aquatic environment, primarily due to enzymatic activity, oxidation, and the growth of harmful microorganisms [3, 4]. Spoilage and pathogenic microorganisms commonly found in seafood, such as Escherichia coli and Staphylococcus aureus, pose serious risks to public health. Globally, food spoilage remains a major concern, with approximately one-quarter of the total food supply—and nearly 30% of fishery products—lost due to microbial contamination [5].

There is increasing interest in using natural methods to preserve food, aiming to maintain quality and freshness while extending shelf life [1, 2]. A variety of natural preservatives derived from animal, plant, and microbial sources have been extensively studied for their potential application in seafood preservation. These include chitosan, essential oils, plant extracts, lactic acid bacteria, bacteriocins, and organic acids [6].

As a result, the food industry is increasingly focusing on the use of various natural extracts from different sources to control and prevent fish diseases and spoilage microorganisms. These efforts aim to enhance fish quality, maintain freshness, extend shelf life, and reduce public health risks. For example, combining citric acid and lactic acid with ice has proven effective in inhibiting bacterial growth and improving the freshness of hake and megrim fillets, ultimately leading to higher market value and better-quality products for consumers [7]. Additionally, the combination of nisin and grape seed extract has demonstrated antibacterial activity against Listeria monocytogenes in ready-to-eat shrimp fillets [8].

Research has shown that compounds derived from Spirulina algae can effectively reduce harmful microorganisms in food, making them suitable for use in fish preservation and as antimicrobial agents [9–11]. Spirulina is considered a valuable natural preservative and antibacterial substance, effective against drug-resistant microbes as well as bacteria and fungi that cause foodborne illnesses. It is widely recognized for its applications as a dietary supplement, a plant-based dye, and a rich source of phenolic compounds and various bioactive secondary metabolites [8, 10]. Owing to its high phycocyanin content, nutritional value, role in the development of functional foods, and therapeutic properties, Spirulina is often regarded as a "superfood" with remarkable health benefits [12, 13].

Spirulina contains bioactive compounds that promote health and help manage non-communicable diseases [16]. These compounds play a crucial role in the prevention and treatment of cardiovascular diseases, high cholesterol, elevated blood sugar, obesity, high blood pressure, tumors, and inflammatory conditions. In addition to strengthening the immune system, Spirulina may help reduce the risk of neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, and multiple sclerosis [17].

Recognized as a natural health solution, Spirulina is widely used in the development of functional foods and dietary supplements due to its well-established health benefits [18, 19]. Its proteins are easily metabolized, making it particularly beneficial for elderly individuals with digestive issues or specific dietary restrictions. Thanks to its rich protein and mineral content, Spirulina can be processed into various forms—including powder, liquid, oil, tablets, and capsules—and incorporated into a wide range of food products. It is commonly used in the production of confectionery, snacks, pastries, and beverages such as fruit juices, which are increasingly valued for their health benefits. Additionally, Spirulina is utilized in the manufacturing of dairy products, pasta, oil-based derivatives, and nutritional supplements [20–22].

As a result, Spirulina has gained significant recognition in scientific communities for its rich nutrient profile, particularly its antioxidant and antimicrobial properties. These compounds function as antioxidants by preventing lipid oxidation and the formation of harmful free radicals, which can negatively affect food flavor and reduce shelf life [23]. However, despite growing research on the use of Spirulina and its extracts as dietary supplements and food preservatives, limited studies have explored their potential as natural preservatives for fish and fishery products.

The objective of this study was to evaluate the antibacterial efficacy of Spirulina platensis extracts on fresh tilapia fillets and to validate the findings using artificial intelligence models such as Artificial Neural Networks (ANN) and Adaptive Neuro-Fuzzy Inference Systems (ANFIS). Specifically, the research investigated the effectiveness of Spirulina platensis extracts at different concentrations in inhibiting Total coliform and Staphylococcus aureus bacteria in fresh tilapia fillets. AI models like ANN and ANFIS were used to simulate and predict the outcomes of the microbiological analysis.

**Materials and Methods  
Extraction and Freezing of *Spirulina***

The harvested blue-green algae Spirulina platensis were imported to Çukurova University in Adana, Türkiye. The prepared Spirulina platensis biomass was stored in sanitary packaging and kept in a freezer at –18°C for 2 hours. The freeze-thaw extraction method, as described in [24], was used to obtain the Spirulina extract, with modifications in concentration. Specifically, 0.5, 1, and 5 grams of freeze-dried Spirulina were each mixed with 100 mL of sterilized distilled water, forming three solution groups labeled as Extract A (EA), Extract B (EB), and Extract C (EC). Maceration of the cells was performed to break down proteins and extract polysaccharides, which are known to exhibit antimicrobial activity against harmful bacteria. The Hu angle was used to evaluate the blue color intensity of the Spirulina extracts [25, 26].

Total coliform and Staphylococcus aureus bacterial strains were selected for this study due to their significant public health and economic importance, especially in the marketing and consumption of fresh products. These bacteria serve as key indicators of the safety and quality of fish and other seafood, as well as the hygiene of water, sanitation practices, and the cleanliness of personnel involved in fish processing. The freeze-thaw extraction method was chosen for its simplicity and the accessibility of the required equipment in our laboratory. This method provides an efficient and cost-effective approach for extracting antimicrobial compounds from Spirulina. A negative control group was included in the experiment, which did not receive any treatment. This control was expected to yield no antimicrobial effect, thereby ensuring that any observed antibacterial activity in the experimental groups could be attributed solely to the Spirulina extract treatment.

Subsequently, 0.5, 1, and 5 grams of Spirulina biomass were carefully measured from the frozen stock and placed into sanitized, labeled bottles. Each bottle was then filled with distilled water up to the 100 mL mark using a volumetric flask. After brief mixing, the bottles were placed in a water bath at 25 °C and covered with aluminum foil to maintain darkness during the 24-hour extraction process. After 24 hours, the supernatant (liquid at the top) was collected and stored at +4 °C.

**Experimental design preparation**

Twenty-five whole Nile tilapia fish (Oreochromis niloticus) were purchased from the Nicosia fish market, then dissected and filleted, yielding 100 fillets weighing approximately 50 grams each. These 100 fillets generated a total of 800 experimental data points. This total comes from multiplying 100 fillets by 4 (three time intervals plus a control group) and 2 bacterial species (Total coliform and S. aureus). Of these, 200 data points were collected during the experimental trial phase and were excluded from the analysis. Therefore, 600 data points were ultimately analyzed.

Sterile plates were used to hold the fish fillets while the extracts were applied to the samples. Initial microbial counts were measured for each fresh fillet and served as controls before treatment with the extracts. The fish fillets were treated with Spirulina extracts at concentrations of 0.5%, 1%, and 5% (w/v), labeled as EA, EB, and EC, respectively. Throughout the experiment, sterile conditions were strictly maintained, with alcohol and flame sterilization used for any equipment contacting the samples.

Microbial analysis was conducted to determine the total viable counts of Total coliform and Staphylococcus aureus before and after treatment with Spirulina platensis extracts. The fillets were stored at 4 °C, and tests were performed at 1, 24, and 48 hours to evaluate the antimicrobial effectiveness of the extracts.

**Analysis of Total Coliform bacteria and *Staphylococcus aureus***

Tilapia fish fillets were weighed in 5 grams samples both before and after treatment. Each sample was placed into a sterile glass jar containing 45 mL of Maximum Recovery Diluent (MRD) and homogenized. Serial 1:10 dilutions were then prepared. Enumeration of Total Coliform bacteria and Staphylococcus aureus was conducted on Plate Count Agar (PCA), with incubation at 37 ± 1°C for 24 ± 2 hours and at 10°C for 7 days. The results were reported as log CFU/g (colony-forming units per gram) [27].

**Analysis of results by artificial intelligence**

According to Metekia et al. (2022), Artificial Neural Networks (ANN) and Adaptive Neuro-Fuzzy Inference Systems (ANFIS) were preferred over conventional statistical techniques for studying the antimicrobial activity of Spirulina platensis because they can overcome the limitations of traditional methods. Unlike linear regression or ANOVA, which assume simple, linear relationships, ANN and ANFIS are capable of modeling complex, non-linear interactions—such as the combined effects of pH, solvent polarity, and extraction time—that are common in biological systems. This allows for more accurate predictions of outcomes, such as inhibition zone size, without relying on predefined equations, capturing dynamic variables that linear models often miss.

Second, in handling complexity and uncertainty, ANN automates the detection of hidden patterns in high-dimensional data (e.g., temperature, solvent ratios), eliminating the need for manual specification of interactions required by conventional methods. ANFIS enhances this capability by integrating fuzzy logic to process subjective or uncertain inputs (e.g., “moderate temperature” or semi-quantitative microbial ratings), effectively bridging qualitative observations with quantitative predictions. This is especially important in biological research, where precise numerical data and strict probabilistic frameworks often fail to capture real-world variability [28].

Third, regarding adaptability and data efficiency, classical models require reconfiguration when new data is introduced, which can hinder iterative optimization (e.g., refining extraction protocols). In contrast, ANN and ANFIS dynamically adapt through training, improving their predictions as new experimental results become available. They also outperform conventional techniques when working with limited or heterogeneous datasets, tolerating noisy inputs and generalizing patterns even from sparse pilot studies, whereas traditional methods typically demand large, homogeneous datasets [28].

Fourth, balancing predictive power and transparency, while the "black box" nature of ANN limits interpretability, ANFIS incorporates fuzzy rules (e.g., “long extraction + polar solvent → increased activity”) to provide greater transparency. This hybrid approach maintains high predictive accuracy while offering insights into the relationships between variables, surpassing purely statistical models. By validating experimental data and enhancing analytical accuracy, ANN and ANFIS streamline the design of antimicrobial studies, accelerating the development of Spirulina-based natural agents [28].

The antibacterial effects of Spirulina algae extracts on Total Coliform bacteria and Staphylococcus aureus in fresh tilapia fish fillets were compared using Artificial Neural Networks (ANN), Adaptive Neuro-Fuzzy Inference Systems (ANFIS), and descriptive statistics. ANFIS, an AI technique, is widely used for predicting various complex problems. It consists of two primary components: a feedforward propagation network and an adaptive multilayer network. The feedforward network utilizes fuzzy Takagi-Sugeno rules to relate input and output variables.

Key elements of the fuzzy inference system include the fuzzifier and defuzzifier. Fuzzy logic membership functions transform crisp input values into fuzzy sets. Nodes representing membership functions model the relationship between inputs and outputs, effectively linking input and output structures. Various membership function types are used, including triangular, sigmoid, Gaussian, and trapezoidal functions [28, 29].

Two primary factors must be considered regarding input and output configurations: the fuzzy inference system (FIS) takes two input variables, 'x' and 'y', and produces one output 'f'. This output is typically modeled using a first-order Sugeno fuzzy rule, which follows a specific mathematical formula.

Method 1: if µ(x) is A1 and µ­y‑ B1 then f1 = p1x + q1y + r1 (1)  
 Method 2: if µ(x) is A2 and µ­y‑ is B2 then f2 = p2x + q2y + r2 (2)

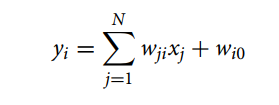
The parameters p1, q1, r1, p2, q2, r2 are outlet function parameters, with constraints A1, B1, A2, B2 being membership functions for x and y. ANFIS is structured with a neural network arrangement consisting of five layers.

The multilayer perceptron (MLP) neural network is a widely used type of artificial neural network (ANN) that is effective for modeling and solving nonlinear systems. Many researchers consider this particular model to be more widely recognized and reliable compared to other forms of ANNs. The structure of the MLP neural network is similar to traditional ANN models, consisting of an input layer, one or more hidden layers, and an output layer. The Levenberg–Marquardt algorithm is commonly used as the learning algorithm to minimize the difference between observed and predicted values. Training is repeated iteratively until the desired level of accuracy is achieved.

The MLP includes input, multiple hidden, and output layers, much like a conventional ANN. In this study, the input data consisted of the concentrations of Spirulina extracts EA, EB, and EC (0.5%, 1%, and 5% w/v), along with the initial microbial load expressed as logCFU/g. The output data represented the antimicrobial activity of the Spirulina extracts and the reduction of microbial counts at 1, 24, and 48 hours (log CFU/g). The flowchart of the study is shown in Figure 1.



**Figure 1.** Flow chart for the experimental study of antimicrobial activity of *S. platensis* extracts on fresh tilapia fish fillets.

**** (3)

Where N is the total number of nodes in the top layer, i; wji is the weight between the nodes i and j in the upper layer; xj represents the output from node j; wi0 is the bias of node i, and yi denotes the input signal to node I, which passes through the transfer function. The data were obtained from laboratory experiments conducted at the Food Hygiene and Technology Department, Veterinary Medicine Faculty of Near East University, Nicosia, Cyprus.

In this analysis, the initial microbial load before treatment (control microbial load, expressed as log₁₀ CFU/g) and the results from each treated sample were used as input variables. The reduction in total bacterial load after treatment at specified time intervals—1, 24, and 48 hours (logCFU/g)—were used as output variables in the analysis and modeling of this study.

**Evaluation criteria for data-driven models:**

The accuracy of any data-driven model is typically assessed by comparing predicted values to observed values. To evaluate the models, the determination of the coefficient (DC) as a measure of goodness of fit, the correlation coefficient (CC), and two statistical error metrics—the root mean squared error (RMSE) and mean squared error (MSE)—were used [28].

(4)

(5)

(6)

MSE = (7)

Where N, Yobsi, Y and Ycomi are data number, observed data, average value of the observed data and computed values, respectively.

**Description and Validation of the Models for the Dataset**

The primary goal of a data-driven approach is to organize data using functional markers within a specific value range to ensure accurate and reliable predictions for unseen datasets. In this process, overfitting and acceptable model performance levels are carefully managed. During the validation phase, various verification methods such as k-fold cross-validation, holdout, and leave-one-out were employed. A key advantage of the k-fold validation method is that both the training and validation sets are automatically determined at each iteration.

As previously mentioned, the dataset was split into two groups: 75% for training and 25% for testing, while also employing k-fold cross-validation. It is important to note that the dataset included 25 instances for each variable.

**Patient and Public Involvement**

This study focused on fresh fish fillets from a food hygiene and safety perspective; therefore, no patients or members of the public were involved.

Code Availability

The custom code and mathematical algorithms developed for the AI-driven analysis in this study, titled "AI Verification for Spirulina's Antimicrobial Power in Total Coliform and Staphylococcus aureus Isolated from Tilapia Fillet," are publicly available to ensure reproducibility and transparency. The code repository is hosted on GitHub/Zenodo/GitHub/Zenodo under the project title supplementary files and can be accessed via the following persistent link: https://www.nature.com/articles/s41598-023-40260-z.

# **Results and Discussion**

**Antimicrobial Activity of Spirulina Extracts on *Total Coliform* and *Staphylococcus aureus***

As shown in Tables 1 and 2, this experimental study evaluated the antimicrobial effects of three different *Spirulina platensis* extracts—EA, EB, and EC—at concentrations of 0.5%, 1%, and 5% (w/v), respectively. Their effectiveness was tested against *Total Coliform* (TC) and *Staphylococcus aureus*, with each extract exhibiting varying levels of antimicrobial efficiency.

For *Total Coliform* (TC), the EA extract reduced the bacterial count from 1.5 log CFU/g (control) to 1.2, 0.9, and 0.4 log CFU/g at 1, 24, and 48 hours, respectively. The EB extract showed a reduction from 1.3 log CFU/g (control) to 0.8, 0.5, and 0.3 log CFU/g over the same time intervals. The EC extract demonstrated the strongest antimicrobial activity, reducing TC counts from 1.4 log CFU/g (control) to 0.8, 0.3, and 0.1 log₀ CFU/g at 1, 24, and 48 hours, respectively (Table 1).

Regarding *S. aureus*, the EA extract decreased the microbial count from 0.57 log CFU/g (control) to 0.44, 0.30, and 0.13 log CFU/g at 1, 24, and 48 hours, respectively. The EB extract reduced counts from 0.71 log CFU/g to 0.26, 0.10, and 0.13 log CFU/g across the same time points. EC again showed the most effective antimicrobial action, reducing *S. aureus* from 0.75 log CFU/g (control) to 0.12, 0.07, and 0.04 log CFU/g at 1, 24, and 48 hours, respectively (Table 2).

As illustrated in Table 1, the one-way ANOVA analysis of the antimicrobial activity of *S. platensis* extracts on *Total Coliform* revealed a significant difference between the control and treatment groups. 95% confidence level is used in this statistical analysis. The F-statistic was 57.29, with a p-value of 3.14 × 10⁻²⁹, which exceeds the F-critical value of 2.64. This indicates that the differences in bacterial reduction among the three extracts were statistically significant (*p < 0.05*).

Similarly, for *S. aureus* (Table 2), the one-way ANOVA showed an F-statistic of 41.2 with a p-value of 2.81 × 10⁻²², also greater than the F-critical value of 2.64. This confirms that the antimicrobial effects of the *Spirulina* extracts on *S. aureus* were statistically significant (*p < 0.05*), validating their potential role in controlling spoilage bacteria in fresh fish fillets.

### Table 1: *Descriptive analysis for S. platensis extracts antimicrobial activity on* Total coliform *bacteria in log CFU/g*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | EA | | | | EB | | | | EC | | | |
|  | C | 1h | 24h | 48h | C | 1h | 24h | 48h | C | 1h | 24 | 48h |
| Mean | 1.5 | 1.2 | 0.9 | 0.4 | 1.3 | 0.8 | 0.5 | 0.3 | 1.4 | 0.8 | 0.3 | 0.1 |
| Standard Error | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 |
| Median | 1.2 | 1.0 | 0.8 | 0.3 | 1.2 | 0.6 | 0.4 | 0.2 | 1.2 | 0.8 | 0.3 | 0.1 |
| Mode | 1.0 | 1.0 | 0.2 | 0.0 | 1.2 | 0.1 | 0.4 | 0.0 | 0.7 | 0.4 | 0.1 | 0.0 |
| Standard Deviation | 0.7 | 0.8 | 0.6 | 0.3 | 0.6 | 0.7 | 0.4 | 0.3 | 0.8 | 0.4 | 0.2 | 0.1 |
| Kurtosis | -0.4 | -0.3 | 0.9 | 2.2 | 0.2 | 0.2 | 1.3 | 0.9 | -0.2 | -0.4 | -1.3 | -1.2 |
| Skewness | 0.9 | 0.6 | 0.8 | 1.2 | 0.0 | 0.9 | 1.1 | 1.1 | 1.1 | 0.5 | 0.4 | 0.5 |
| Range | 2.8 | 2.8 | 2.4 | 1.4 | 2.7 | 2.5 | 1.7 | 1.0 | 2.4 | 1.5 | 0.7 | 0.4 |
| Minimum | 0.5 | 0.2 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.6 | 0.1 | 0.1 | 0.0 |
| Maximum | 3.3 | 3.0 | 2.5 | 1.4 | 2.8 | 2.5 | 1.7 | 1.0 | 3.0 | 1.6 | 0.8 | 0.4 |

### Table 2: *Descriptive statistic for S. platensis extracts antimicrobial activity on* *Staphylococci bacteria descriptive statistic in log CFU/g*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SA – EA | | | | | SA – EB | | | | SA- EC | | | |
|  | C | 1h | 24h | 48h | C | 1h | 24h | 48h | C | 1h | 24h | 48h |
| Mean | 0.57 | 0.44 | 0.3 | 0.13 | 0.71 | 0.26 | 0.1 | 0.13 | 0.75 | 0.12 | 0.07 | 0.04 |
| Standard Error | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| Median | 0.4 | 0.4 | 0.2 | 0.1 | 0.6 | 0.2 | 0.1 | 0.0 | 0.9 | 0.1 | 0.0 | 0.0 |
| Mode | 1.6 | 0.1 | 0.0 | 0.0 | 1.8 | 0.2 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Standard Deviation | 0.6 | 0.4 | 0.3 | 0.1 | 0.6 | 0.2 | 0.1 | 0.1 | 0.4 | 0.1 | 0.1 | 0.1 |
| Kurtosis | -0.6 | -0.9 | -0.4 | 1.3 | -0.3 | -0.3 | -0.2 | -0.1 | 0.0 | -0.5 | -0.5 | 1.1 |
| Skewness | 1.2 | 0.5 | 0.7 | 1.2 | 1.0 | 0.8 | 0.9 | 1.1 | 0.2 | 0.8 | 0.8 | 1.4 |
| Range | 1.5 | 1.2 | 0.9 | 0.5 | 1.8 | 0.7 | 0.5 | 0.3 | 1.6 | 0.4 | 0.2 | 0.2 |
| Minimum | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Maximum | 2 | 1.2 | 0.9 | 0.5 | 1.8 | 0.7 | 0.5 | 0.3 | 2 | 0.4 | 0.2 | 0.2 |

The antimicrobial activity of Spirulina platensis extracts against Total Coliform and Staphylococcus aureus was further illustrated through line graphs, bar charts, and radar charts (Figs. 2 and 3). All three extracts—EA, EB, and EC—demonstrated effective antimicrobial properties, with EC exhibiting the strongest activity compared to the others.

### Figure 2: *Antimicrobial activity of Spirulina extracts on TC and S. aureus bacteria using bar chart (log CFU/g)*

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### Total coliform *S. aureus*

### Figure 3: *Antimicrobial activity of Spirulina extracts on Total coliform and S. aureus using radar chart (log CFU/g)*

A study conducted on fresh tilapia fish (Oreochromis niloticus) in Kenya reported that some samples exceeded the acceptable limit of 5.00 log CFU/g for total viable count. Additionally, the majority of the fish samples had total coliform and fecal coliform counts above 2.00 and 1.00 log CFU/g, respectively [30]. These findings support the current study, which demonstrated that Spirulina platensis extracts effectively control and inhibit the growth of food spoilage and pathogenic bacteria, including both Gram-positive and Gram-negative strains such as E. coli and S. aureus [10, 31].

Another study identified peptides in S. platensis composed of 18 amino acids that exhibited antibacterial activity against E. coli and S. aureus, with minimum inhibitory concentrations (MICs) of 8 mg/mL and 16 mg/mL, respectively [32].

Additional supportive evidence comes from research showing that a 1% (w/v) concentration of microalgae extracts (including Spirulina platensis and Chlorella vulgaris) effectively suppressed bacterial growth and extended the shelf life of vacuum-packed, chilled sardines by three days. Among the two, Spirulina platensis demonstrated superior antibacterial efficacy [33]. In a related trial, the MIC values of Spirulina extracts against E. coli and S. aureus were again found to be 8 mg/mL and 16 mg/mL, respectively, and the extracts were confirmed to be non-toxic [32].

Incorporating a portion of Spirulina biomass into food products can serve as a natural additive, enhancing nutritional value and providing antioxidant benefits. This inclusion allows products to achieve an extended shelf life of up to 45 days without the need for synthetic preservatives [14]. Research focused on older adults reported that chocolate-flavored powder shakes enriched with Spirulina had a shelf life of up to 19 months post-production. These shakes were consumed for their high protein and carbohydrate content, offering essential energy and nutrients for elderly individuals [15].

Further supporting evidence comes from a recent study by Selim et al. (2025), which demonstrated the antibacterial activity of Spirulina algal extract. This effect is attributed to its rich profile of bioactive compounds, including polyunsaturated fatty acids (PUFAs), polysaccharides, glycosides, peptides, flavonoids, phycocyanin, minerals, essential amino acids, and vitamins. The study used both in vitro assays (such as the crystal violet assay and qRT-PCR) and an in vivo murine pneumonia model. Results showed that the extract's efficacy was comparable to colistin, improving lung structure, reducing inflammatory cell infiltration, and minimizing fibrosis, as confirmed by histological and immunohistochemical analysis. These findings position Arthrospira maxima as a promising candidate for novel antibacterial therapies [44].

Additionally, other studies have reinforced the antimicrobial role of bioactive compounds from Spirulina platensis, such as PUFAs, polysaccharides, glycosides, peptides, and neophytadiene, in combating pathogenic microorganisms [42, 43].

**ANN and ANFIS Model Analysis of Spirulina platensis Extracts on Total Coliform and S. aureus (logCFU/g)**

The antimicrobial activity of S. platensis extracts (EA, EB, and EC) against Total Coliform (TC) and Staphylococcus aureus was analyzed using Artificial Neural Network (ANN) and Adaptive Neuro-Fuzzy Inference System (ANFIS) models, with results expressed in log CFU/g.

The ANN model simulated the antimicrobial effect of Spirulina extracts on TC at 1, 24, and 48 hours. For EA, the predicted microbial loads were 1.36, 0.99, and 0.44 log CFU/g, respectively. For EB, the values were 0.96, 0.58, and 0.32 log CFU/g, while EC exhibited the strongest antimicrobial performance, with predicted values of 0.74, 0.38, and 0.16 log₁₀ CFU/g at the corresponding time intervals. Among the three extracts, EC consistently demonstrated superior antimicrobial efficacy. The ANN model achieved high correlation coefficients (R-values) of 0.95817 for training, 0.91795 for testing, and 0.94829 for validation (see Table 3).

Similarly, the ANFIS model simulated the antimicrobial effect of EA, EB, and EC on TC. The predicted bacterial counts for EA were 0.42, 0.39, and 0.33 log CFU/g at 1, 24, and 48 hours, respectively. For EB, the values were 0.35, 0.32, and 0.32 log CFU/g, while EC achieved 0.42, 0.24, and 0.18 log CFU/g at the same time points. These findings, presented in Table 4, further confirm EC’s superior antimicrobial activity compared to EA and EB.

The antimicrobial effects of S. platensis extracts (EA, EB, and EC) on Staphylococcus aureus were evaluated at 1, 24, and 48 hours. EA reduced S. aureus counts from 0.57 log CFU/g (control) to 0.44, 0.30, and 0.13 log CFU/g, respectively. EB showed reductions from 0.71 log CFU/g to 0.26, 0.10, and 0.13 log CFU/g, while EC achieved the most pronounced effects, decreasing counts from 0.75 log CFU/g to 0.12, 0.07, and 0.04 log CFU/g, respectively.

The ANN model simulated S. platensis extract performance over time, predicting S. aureus counts of 0.49, 0.32, and 0.16 log CFU/g for EA at 1, 24, and 48 hours, respectively. EB values were predicted at 0.29, 0.17, and 0.10 log CFU/g, while EC recorded the lowest predicted values at 0.01, 0.002, and 0.001 log CFU/g for the same intervals. Regression coefficients (R-values) for EA were 0.9532 (training), 0.9695 (validation), and 0.9163 (testing). EB showed R-values of 0.9534, 0.8879, and 0.9458, while EC exhibited the strongest performance with R-values of 0.9209, 0.9512, and 0.9156, respectively (see Table 5). Model performance metrics including MSE and RMSE can be found in Table 7.

Similarly, ANFIS modeling showed average predicted bacterial loads of 0.43, 0.30, and 0.12 log₁₀ CFU/g for EA, and 0.31, 0.16, and 0.09 log CFU/g for EB at 1, 24, and 48 hours, respectively. EC predictions were 0.36, 0.18, and 0.07 log CFU/g. The regression results for EA were R = 0.9167, 0.9258, and 0.9093. EB showed R = 0.9591, 0.9158, and 0.9116, while EC achieved the highest model accuracy with R = 0.9893, 0.9763, and 0.9711 (see Table 6). Corresponding MSE and RMSE values are provided in Table 8.

Several supporting studies reinforce the utility of ANN and ANFIS models in microbiological prediction. For instance, ANN has been used to model the effects of pH, ethanol, and salt concentrations on S. aureus biofilm formation, with successful inhibition under high ethanol and salt levels at 24 and 48 hours of incubation at 37°C [34]. ANN has also been applied to predict seafood storage time using colorimetric standards [35], and to evaluate the antimicrobial action of natural extracts from Zataria multiflora against S. aureus [36].

Other studies show ANN's effectiveness in forecasting antimicrobial compounds effective against E. coli [37], and in predicting total coliform levels in foods using image recognition—results that correlate well with the traditional multiple-tube fermentation method [38]. Similarly, ANFIS has been employed to predict the presence of total coliforms, E. coli, intestinal enterococci, and Clostridium perfringens in untreated water samples with high accuracy [39].

In a separate study, ANFIS successfully modeled the inactivation of S. aureus using ultrasonic treatment, reporting a peak efficacy at an amplitude of 37.5 µm with an R-value of 0.979 [40]. Additionally, a Turkish study demonstrated that a 10% concentration of Gamay grape powder eliminated S. aureus by the fifth day of treatment, with the ANFIS model outperforming both ANN and multiple linear regression (MLR) models in predictive accuracy [41].

### Table 3: *S. platensis extracts antimicrobial activity on TC bacteria modeling using ANN model and results are in log CFU/g*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Training Stage – TC | | | | | | | | | | |
| EA | | | EB | | | | EC | | | |
| 1h | 24h | 48h | | 1h | 24h | 48h | | 1h | 24h | 48h | |
| 0.5595 | 0.4410 | 0.1968 | | 0.4450 | 0.2413 | 0.1346 | | 0.3616 | 0.1078 | 0.0285 | |
| 0.5595 | 0.4410 | 0.1968 | | 0.4450 | 0.2413 | 0.1346 | | 0.3616 | 0.1078 | 0.0285 | |
| 0.5595 | 0.4410 | 0.1968 | | 0.4450 | 0.2413 | 0.1346 | | 0.3616 | 0.1078 | 0.0285 | |
| 0.5595 | 0.4410 | 0.1968 | | 0.4450 | 0.2413 | 0.1346 | | 0.3616 | 0.1078 | 0.0285 | |
| 0.5595 | 0.4410 | 0.1968 | | 0.4450 | 0.2413 | 0.1346 | | 0.3616 | 0.1078 | 0.0285 | |
| 0.9259 | 0.6530 | 0.2622 | | 0.7352 | 0.3146 | 0.1592 | | 1.3568 | 0.3423 | 0.1163 | |
| 1.1845 | 0.8026 | 0.3082 | | 0.5858 | 0.2760 | 0.1460 | | 1.3674 | 0.3375 | 0.1134 | |
| 0.5224 | 0.4190 | 0.1897 | | -0.1702 | -0.0324 | 0.0058 | | 1.4558 | 0.2972 | 0.0893 | |
| 0.5193 | 0.4170 | 0.1889 | | -0.2250 | -0.0533 | -0.0035 | | 1.0458 | 0.4841 | 0.2011 | |
| 2.0522 | 1.4044 | 0.5230 | | 0.1768 | 0.1005 | 0.0646 | | 1.1792 | 0.4233 | 0.1647 | |
| 0.4544 | 0.3310 | 0.1322 | | 1.1294 | 0.7269 | 0.4067 | | 0.3571 | 0.1038 | 0.0265 | |
| 1.6866 | 1.2205 | 0.5835 | | 1.4875 | 0.5225 | 0.2859 | | 0.8547 | 0.5695 | 0.2522 | |
| 0.5100 | 0.4089 | 0.1847 | | 0.7352 | 0.3146 | 0.1592 | | 0.6686 | 0.4004 | 0.1705 | |
| 0.8835 | 0.6285 | 0.2546 | | 0.7361 | 0.3150 | 0.1595 | | 0.3590 | 0.1055 | 0.0273 | |
| 1.6944 | 1.2303 | 0.5925 | | 1.1294 | 0.7269 | 0.4067 | | 0.3636 | 0.1096 | 0.0293 | |
| 0.6191 | 0.4756 | 0.2076 | | 1.1386 | 0.8183 | 0.4533 | | 0.8614 | 0.5679 | 0.2512 | |
| 0.5181 | 0.4162 | 0.1886 | | 1.0805 | 0.7775 | 0.4349 | | 0.7788 | 0.5051 | 0.2213 | |
| Testing Stage – TC | | | | | | | | | | |
| 1.6123 | 1.1249 | 0.4939 | | 0.7298 | 0.3137 | 0.1591 | | 0.8166 | 0.5404 | 0.2384 | |
| 1.9073 | 1.4551 | 0.7631 | | 1.2476 | 0.7151 | 0.3773 | | 0.8400 | 0.5610 | 0.2483 | |
| 2.1559 | 1.3175 | 0.2624 | | 0.6259 | 0.2864 | 0.1496 | | 0.8699 | 0.5642 | 0.2490 | |
| 0.8049 | 0.5831 | 0.2407 | | 2.5433 | 1.7037 | 0.9982 | | 0.7700 | 0.4968 | 0.2172 | |
| 2.0075 | 1.4420 | 0.6356 | | 1.4451 | 0.8985 | 0.4801 | | 0.8166 | 0.5404 | 0.2384 | |
| 2.9601 | 2.4594 | 1.4017 | | 1.0817 | 0.7722 | 0.4321 | | 0.6988 | 0.4292 | 0.1844 | |
| 1.9073 | 1.4551 | 0.7631 | | 1.2474 | 0.8510 | 0.4652 | | 0.3560 | 0.1029 | 0.0261 | |
| 1.5232 | 1.0087 | 0.3836 | | 1.3533 | 0.8778 | 0.4740 | | 0.8066 | 0.5311 | 0.2339 | |

### Table 4: *S. platensis extracts antimicrobial activity on TC bacteria modeling using ANFIS Model and results are in log CFU/g*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Training Stage- TC | | | | | | | | |
| EA | | | EB | | | EC | | |
| 1H | 24H | 48H | 1H | 24H | 48H | 1H | 24H | 48H |
| 0.0421 | 0.0843 | -0.0668 | 0.1032 | 0.0187 | 0.0011 | 0.1326 | 0.0872 | 0.0114 |
| 0.0724 | 0.0722 | 0.1095 | 0.0414 | 0.1106 | 0.0172 | 0.0806 | 0.0634 | 0.0272 |
| 0.0765 | 0.0831 | 0.1212 | 0.0647 | 0.0704 | 0.0404 | 0.1326 | 0.0872 | 0.0114 |
| 0.0733 | 0.0731 | 0.1154 | 0.0324 | 0.0408 | 0.1974 | 0.1043 | 0.0405 | 0.0092 |
| 0.1922 | 0.186 | 0.1752 | 0.0699 | 0.2214 | 0.0381 | 0.0785 | 0.0564 | 0.0261 |
| 0.2612 | 0.2385 | 0.1948 | 0.2015 | 0.1344 | 0.1247 | 0.9918 | 0.3127 | 0.1499 |
| 0.2846 | 0.2555 | 0.201 | 0.3209 | 0.3031 | 0.3488 | 0.9749 | 0.31 | 0.0795 |
| 0.1691 | 0.1716 | 0.1697 | 0.0001 | 0.0024 | 0.0435 | 0.9205 | 0.5103 | 0.3615 |
| 0.149 | 0.1546 | 0.1624 | 0.0002 | 0.0004 | 0.0048 | 0.6626 | 0.4552 | 0.3804 |
| 0.6432 | 0.5704 | 0.4818 | 0.0451 | 0.0255 | 0.0693 | 0.6158 | 0.5808 | 0.3504 |
| 0.0135 | 0.0602 | 0.1259 | 0.1193 | 0.3735 | 0.3311 | 0.1476 | 0.1529 | 0.1513 |
| 0.5513 | 0.4764 | 0.3505 | 0.7159 | 0.3891 | 0.3443 | 0.5614 | 0.0155 | 0.1608 |
| 0.2874 | 0.2839 | 0.236 | 0.2015 | 0.1344 | 0.1247 | 0.3805 | 0.1674 | 0.0986 |
| 0.2573 | 0.2356 | 0.1937 | 0.2091 | 0.1275 | 0.1484 | 0.0962 | 0.1071 | 0.0564 |
| 0.5479 | 0.469 | 0.3576 | 0.269 | 0.1601 | 0.1583 | 0.2477 | 0.0147 | 0.0374 |
| 0.2225 | 0.2103 | 0.1843 | 0.6572 | 0.6532 | 0.7197 | 0.7234 | 1.0852 | 0.9974 |
| 0.1577 | 0.1635 | 0.1666 | 0.5334 | 0.5542 | 0.5157 | 0.4662 | 0.8236 | 0.7735 |
| Testing Stage- TC | | | | | | | | |
| 0.4722 | 0.471 | 0.6443 | 0.15 | 0.1217 | 0.1752 | 0.5115 | 0.3991 | 0.38059 |
| 0.5623 | 0.5023 | 0.4638 | 0.4131 | 0.4134 | 0.2492 | 0.4822 | 0.3274 | 0.31535 |
| 0.774 | 0.6233 | 0.3224 | 0.3905 | 0.2066 | 0.1926 | 0.3903 | 0.0496 | 0.03601 |
| 0.2398 | 0.2208 | 0.1891 | 0.9998 | 1 | 1 | 0.47 | 0.1795 | 0.1404 |
| 0.72 | 0.6439 | 0.5396 | 0.5309 | 0.5065 | 0.5025 | 0.3655 | 0.2131 | 0.1167 |
| 1.0054 | 0.9816 | 0.9134 | 0.245 | 0.2986 | 0.3715 | 0.3626 | 0.2075 | 0.1024 |
| 0.6244 | 0.5418 | 0.429 | 0.551 | 0.5211 | 0.5267 | 0.1931 | 0.09467 | 0.03905 |
| 0.4685 | 0.4027 | 0.2102 | 0.5581 | 0.5276 | 0.5408 | 0.4161 | 0.1437 | 0.03093 |

### Table 5: *S. platensis extracts antimicrobial activity on S. aureus bacteria modeling using ANN model and results are in log CFU/g*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Training Stage – *Staphylococci* | | | | | | | | |
| EA | | | EB | | | EC | | |
| 1h | 24h | 48h | 1h | 24h | 48h | 1h | 24h | 48h |
| 0.0374 | 0.0040 | 0.0188 | 0.1907 | 0.0011 | 0.0002 | 0.0115 | 0.0060 | 0.0020 |
| 0.1236 | 0.0720 | 0.0047 | 0.1600 | 0.0724 | 0.0111 | 0.0115 | 0.0060 | 0.0020 |
| 0.1648 | 0.0982 | 0.0215 | 0.1556 | 0.0835 | 0.0114 | 0.0114 | 0.0060 | 0.0021 |
| 0.1434 | 0.0846 | 0.0128 | 0.1193 | 0.0008 | 0.0004 | 0.0115 | 0.0060 | 0.0020 |
| 0.4395 | 0.2723 | 0.1421 | 0.1907 | 0.0011 | 0.0002 | 0.0115 | 0.0060 | 0.0020 |
| 0.4613 | 0.2769 | 0.1618 | 0.1764 | 0.0850 | 0.0530 | 0.0006 | 0.0002 | 0.0004 |
| 0.1491 | 0.0892 | 0.0256 | 0.1162 | 0.0560 | 0.0237 | 0.0068 | 0.0037 | 0.0014 |
| 0.1469 | 0.0878 | 0.0246 | 0.2550 | 0.1474 | 0.0881 | 0.0009 | 0.0003 | 0.0002 |
| 0.6215 | 0.3729 | 0.2321 | 0.2028 | 0.1067 | 0.0654 | 0.0005 | 0.0001 | -0.0002 |
| 0.1553 | 0.0929 | 0.0282 | 0.4170 | 0.2821 | 0.1593 | 0.0045 | 0.0025 | 0.0011 |
| 0.1458 | 0.0872 | 0.0240 | 0.0765 | 0.0236 | 0.0059 | 0.0012 | 0.0005 | 0.0002 |
| 0.1075 | 0.0636 | 0.0453 | 0.0801 | 0.0089 | 0.0099 | 0.0026 | 0.0015 | 0.0007 |
| 0.9238 | 0.7068 | 0.3713 | 0.0515 | 0.0133 | 0.0067 | 0.0018 | 0.0009 | 0.0004 |
| 1.1929 | 0.8344 | 0.5152 | 0.0797 | 0.0085 | 0.0097 | 0.0019 | 0.0010 | 0.0005 |
| 0.6779 | 0.4681 | 0.4794 | 0.0797 | 0.0085 | 0.0097 | 0.0025 | 0.0015 | 0.0009 |
| 0.601 | 0.361 | 0.223 | 0.483 | 0.320 | 0.189 | 0.001 | 0.001 | 0.001 |
| 0.697 | 0.496 | 0.101 | 0.255 | 0.147 | 0.088 | 0.003 | 0.002 | 0.001 |
| Testing Stage – *Staphylococci* | | | | | | | | |
| 0.981 | 0.674 | 0.091 | 0.222 | 0.144 | 0.071 | 0.001 | 0.000 | 0.000 |
| 0.443 | 0.262 | 0.157 | 0.113 | 0.035 | 0.024 | 0.003 | 0.001 | 0.001 |
| 0.465 | 0.275 | 0.166 | 0.358 | 0.208 | 0.139 | 0.002 | 0.001 | 0.000 |
| 0.430 | 0.258 | 0.148 | 0.541 | 0.402 | 0.210 | 0.004 | 0.002 | 0.001 |
| 0.281 | 0.168 | 0.083 | 0.539 | 0.338 | 0.222 | 0.002 | 0.001 | 0.000 |
| 0.543 | 0.328 | 0.202 | 0.400 | 0.267 | 0.154 | 0.002 | 0.001 | 0.001 |
| 0.507 | 0.306 | 0.185 | 0.488 | 0.319 | 0.191 | 0.001 | 0.000 | 0.000 |
| 0.919 | 0.668 | 0.299 | 0.468 | 0.307 | 0.182 | 0.003 | 0.002 | 0.001 |

### Table 6: *S. platensis extracts antimicrobial activity on Staphylococci bacteria modeling using ANFIS model and results are in log CFU/g*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ANFIS Training Stage – *Staphylococci* | | | | | | | | |
| EA | | | EB | | | EC | | |
| 1h | 24h | 48h | 1h | 24h | 48h | 1h | 24h | 48h |
| 0.0319 | 0.0519 | 0.0290 | 0.1286 | 0.0000 | 0.0000 | 1.2037 | 0.8272 | 0.0425 |
| 0.0755 | 0.0218 | 0.0075 | 0.1553 | 0.0000 | 0.0000 | 1.1748 | 0.0535 | 0.0232 |
| 0.1706 | 0.2361 | 0.0909 | 0.1676 | 0.0000 | 0.0000 | 1.3000 | 0.0783 | 0.0800 |
| 0.1280 | 0.0271 | 0.0082 | 0.1200 | 0.0000 | 0.0000 | 1.1882 | 0.9514 | 0.4331 |
| 0.0518 | 0.0850 | 0.0168 | 0.1286 | 0.0000 | 0.0000 | 1.1324 | 0.6450 | 0.0389 |
| 0.2878 | 0.1634 | 0.0832 | 0.2257 | 0.1290 | 0.0738 | 0.0411 | 0.0485 | 0.0085 |
| 0.1100 | 0.0616 | 0.0258 | 0.1142 | 0.0680 | 0.0301 | 0.5853 | 0.4321 | 0.2232 |
| 0.0878 | 0.0489 | 0.0187 | 0.2996 | 0.1629 | 0.1208 | 0.0476 | 0.0151 | 0.0205 |
| 0.3168 | 0.1804 | 0.1019 | 0.2106 | 0.1599 | 0.0909 | 0.0077 | 0.0274 | 0.0332 |
| 0.1290 | 0.0721 | 0.0349 | 0.4439 | 0.2150 | 0.1170 | 0.4277 | 0.2794 | 0.0219 |
| 0.0656 | 0.0362 | 0.0115 | 0.0680 | 0.0159 | 0.0220 | 0.0963 | 0.0151 | 0.0149 |
| 0.1716 | 0.1014 | 0.0512 | 0.0522 | 0.0018 | 0.0095 | 0.2924 | 0.1930 | 0.0876 |
| 0.9480 | 0.6950 | 0.3586 | 0.0584 | 0.0166 | 0.0270 | 0.1913 | 0.0885 | 0.0743 |
| 1.0871 | 0.8228 | 0.4054 | 0.0482 | 0.0021 | 0.0080 | 0.2091 | 0.1039 | 0.0119 |
| 0.9276 | 0.6100 | 0.3091 | 0.0482 | 0.0021 | 0.0080 | 0.2788 | 0.1590 | 0.1805 |
| 0.3277 | 0.2925 | 0.0961 | 0.5152 | 0.2634 | 0.0212 | 0.1431 | 0.0773 | 0.0987 |
| 0.6925 | 0.4812 | 0.1412 | 0.2996 | 0.1629 | 0.0908 | 0.3454 | 0.2142 | 0.1193 |
| ANFIS Testing Stage – *Staphylococci* | | | | | | | | |
| 0.9276 | 0.6053 | 0.0248 | 0.2190 | 0.1614 | 0.0701 | 0.0637 | 0.0382 | 0.0238 |
| 0.4325 | 0.2462 | 0.1275 | 0.1375 | 0.0794 | 0.0493 | 0.2183 | 0.1091 | 0.0432 |
| 0.6588 | 0.3786 | 0.1447 | 0.3444 | 0.1781 | 0.1038 | 0.1623 | 0.0816 | 0.0349 |
| 0.2636 | 0.3576 | 0.0895 | 0.5396 | 0.2638 | 0.1417 | 0.3851 | 0.1798 | 0.1120 |
| 0.2389 | 0.1354 | 0.0674 | 0.5735 | 0.4745 | 0.2604 | 0.1474 | 0.0712 | 0.0430 |
| 0.4631 | 0.2826 | 0.2457 | 0.4153 | 0.2685 | 0.2489 | 0.2739 | 0.1661 | 0.0145 |
| 0.4530 | 0.2793 | 0.1435 | 0.7221 | 0.3604 | 0.2125 | 0.0558 | 0.0167 | 0.0201 |
| 0.8605 | 0.6091 | 0.2314 | 0.4847 | 0.2415 | 0.1157 | 0.3275 | 0.1870 | 0.0409 |

### *Table 7:* *ANN model MSE and RMSE in the simulations of S. platensis extracts antimicrobial activity over Total Coliform and Staphylococcus aureus modeling along the time duration*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Training | | | | | | |
|  | MSE | | | RMSE | | |
|  | 1h | 24h | 48h | 1h | 24h | 48h |
| ANN-TC-EA | 0.0549 | 0.0385 | 0.0247 | 0.2344 | 0.1962 | 0.1572 |
| ANN-TC-EB | 0.1074 | 0.0309 | 0.0172 | 0.3277 | 0.1757 | 0.1313 |
| ANN-TC-EC | 0.0261 | 0.0268 | 0.0055 | 0.1616 | 0.1638 | 0.0745 |
| ANN-SA-EA | 0.0193 | 0.0088 | 0.0049 | 0.1390 | 0.0939 | 0.0701 |
| ANN-SA-EB | 0.0028 | 0.0024 | 0.0021 | 0.0527 | 0.0489 | 0.0459 |
| ANN-SA-EC | 0.4741 | 0.1510 | 0.0243 | 0.6885 | 0.3885 | 0.1558 |
| Testing | | | | | | |
| ANN-TC-EA | 0.0451 | 0.0314 | 0.0304 | 0.2124 | 0.1773 | 0.1744 |
| ANN-TC-EB | 0.0592 | 0.0102 | 0.0146 | 0.2434 | 0.1008 | 0.1209 |
| ANN-TC-EC | 0.0061 | 0.0143 | 0.0045 | 0.0779 | 0.1195 | 0.0673 |
| ANN-SA-EA | 0.0111 | 0.0045 | 0.0026 | 0.1056 | 0.0674 | 0.0510 |
| ANN-SA-EB | 0.0078 | 0.0086 | 0.0032 | 0.0885 | 0.0925 | 0.0563 |
| ANN-SA-EC | 0.0489 | 0.0116 | 0.0038 | 0.2211 | 0.1078 | 0.0617 |

*Table 8. ANFIS model MSE and RMSE in the simulation of S. platensis extracts antimicrobial activity over Total Coliform and Staphylococcus aureus modeling along the time duration*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Training | | | | | | |
|  | MSE | | | RMSE | | |
|  | 1h | 24h | 48h | 1h | 24h | 48h |
| ANFIS-TC-EA | 0.5099 | 0.2149 | 0.0221 | 0.7141 | 0.4635 | 0.1487 |
| ANFIS-TC-EB | 0.2424 | 0.0445 | 0.0029 | 0.4923 | 0.2109 | 0.0535 |
| ANFIS-TC-EC | 0.1511 | 0.0184 | 0.0523 | 0.3887 | 0.1358 | 0.2288 |
| ANFIS-SA-EA | 0.0022 | 0.0014 | 0.0006 | 0.0465 | 0.0374 | 0.0239 |
| ANFIS-SA-EB | 0.0022 | 0.0178 | 0.0006 | 0.0465 | 0.1333 | 0.0239 |
| ANFIS-SA-EC | 0.0019 | 0.0021 | 0.0008 | 0.0437 | 0.0457 | 0.0291 |
| Testing | | | | | | |
| ANFIS-TC-EA | 1.9045 | 0.8359 | 0.0846 | 1.3800 | 0.9143 | 0.2908 |
| ANFIS-TC-EB | 0.7768 | 0.1511 | 0.0094 | 0.8813 | 0.3887 | 0.0967 |
| ANFIS-TC-EC | 0.1274 | 0.1345 | 0.0177 | 0.3570 | 0.3667 | 0.1332 |
| ANFIS-SA-EA | 0.0002 | 0.0021 | 0.0009 | 0.0141 | 0.0462 | 0.0301 |
| ANFIS-SA-EB | 0.0002 | 0.0021 | 0.0009 | 0.0141 | 0.0462 | 0.0301 |
| ANFIS-SA-EC | 0.0018 | 0.0009 | 0.0002 | 0.0422 | 0.0306 | 0.0155 |

**Comparison with Standard Preservatives**

As previously discussed, this experimental study evaluated the antimicrobial efficacy of three Spirulina platensis extracts—EA, EB, and EC—at concentrations of 0.5%, 1%, and 5% (w/v), respectively, against Total Coliform (TC) and Staphylococcus aureus. The results demonstrated a clear concentration- and time-dependent antimicrobial effect. Among the tested extracts, EC (5% w/v) exhibited the highest antimicrobial activity, reducing TC levels from 1.4 to 0.1 log CFU/g and S. aureus levels from 0.75 to 0.04 log CFU/g after 48 hours.

The superior performance of EC is attributed to its higher concentration of bioactive compounds, particularly polyphenols and phycocyanin, which are known to disrupt bacterial cell membranes and interfere with metabolic processes. In the case of S. aureus, compounds such as benzophenone and isoquinoline may also contribute to the antimicrobial effect by inhibiting bacterial enzymes and compromising membrane integrity.

Although this study did not directly compare Spirulina extracts with synthetic preservatives like sodium benzoate or potassium sorbate, insights from existing literature provide useful context:

* **Natural Preservatives**: The antimicrobial efficacy of Spirulina aligns with or surpasses other natural agents. For instance, Arthrospira platensis extracts at a 0.9% concentration demonstrated bactericidal effects against Listeria monocytogenes in salmon tartare, comparable to the commonly used bacteriocin, nisin [45].
* **Synthetic Preservatives**: Sodium benzoate typically achieves bacterial load reductions of 1–2 log CFU/g in food systems, depending on pH and concentration. In this study, Spirulina EC extract achieved a 1.3 log CFU/g reduction in TC and a 0.71 log CFU/g reduction in S. aureus, suggesting a comparable—though slightly lower—efficacy under the tested conditions. Unlike synthetic preservatives, however, Spirulina also offers antioxidant properties, nutritional value, and low toxicity, making it a multifunctional alternative [43, 45–48].

While this study provides strong evidence for Spirulina’s potential as a natural antimicrobial agent, direct comparisons with synthetic preservatives remain inferred rather than empirically tested. Nevertheless, EC (5% w/v) showed the strongest antimicrobial activity and could serve as a promising natural preservative in food systems, particularly when used in combination with other natural agents to enhance overall efficacy.

For practical applications, Spirulina’s antimicrobial performance appears comparable to mid-range synthetic preservatives but with added nutritional and health benefits.

**Conclusion and Recommendations**

This research demonstrated the effectiveness of Spirulina platensis extracts—EA, EB, and EC—in reducing the counts of Total Coliform and Staphylococci bacteria in fresh fish fillets. This conclusion is supported by detailed analyses using descriptive statistics, as well as Artificial Neural Network (ANN) and Adaptive Neuro-Fuzzy Inference System (ANFIS) models. Notably, significant differences were observed between the initial bacterial load (control group) and the treated samples across all experiments. Both ANN and ANFIS models performed well in predicting microbial reduction, indicating the value of AI-based methods in food safety research.

Among the extracts, EC (5% w/v) showed the most effective antimicrobial activity, followed by EB (1% w/v) and EA (0.5% w/v). These findings suggest that the antimicrobial effectiveness of Spirulina platensis increases with concentration, making it more efficient at controlling spoilage microorganisms in fresh fish fillets. Both ANN and ANFIS models provided accurate predictions regarding the antimicrobial effects of varying concentrations of S. platensis on Total Coliform and Staphylococci.

S. platensis, a sustainable source of bioactive compounds, holds promise as a natural preservative for fish fillets and potentially other food products. Future research should focus on identifying and analyzing the specific bioactive compounds responsible for these effects and their impact on various spoilage microorganisms in foods such as poultry, raw meat, and milk.

Given that the antimicrobial activity of Spirulina is attributed to its chemical composition and mechanisms of action, future studies should also aim to identify the most effective techniques for extracting these bioactive compounds. Additionally, the potential risks of wild-harvested Spirulina, such as contamination with heavy metals and other toxic substances, must be thoroughly evaluated to ensure safety for human consumption and biotechnological use.

More broadly, promoting the use of natural preservatives like Spirulina can play a key role in improving food safety and public health. This can be achieved by:

* Encouraging its inclusion in food processing;
* Educating consumers about the benefits of consuming Spirulina-treated tilapia fillets;
* Supporting eco-friendly and sustainable food preservation practices.

To fully harness the antimicrobial properties of *Spirulina platensis* across seafood, animal-derived products, and fresh juices and vegetables, the following strategic recommendations are proposed:

Advanced Analytical Approaches**:** Employ state-of-the-art techniques such as metabolomics, genomics, and proteomics to isolate and characterize the specific bioactive antimicrobial compounds in *Spirulina*. These methods will also aid in understanding their mechanisms of action at the molecular level.

Optimized Extraction Methods**:** Develop efficient and scalable extraction techniques that preserve the bioactivity of *Spirulina* compounds. This will enhance the antimicrobial efficacy of extracts and support their stable incorporation into various food systems.

Innovative Formulation Strategies**:** Investigate and design novel food formulation methods that allow seamless integration of *Spirulina* extracts into a wide range of food matrices. These strategies should prioritize compound stability, bioavailability, and prolonged antimicrobial action.

Support for Local Application in Fish Processing and Value Addition**:** To promote the use of *S. platensis* extract among fishermen, fish processors, and local consumers, we further recommend:

* Nutritional and Functional Evaluation: Conduct comprehensive nutritional profiling and assess the value addition of fish fillets enriched with *Spirulina*-derived phenolic compounds.
* Application of AI-based Predictive Models: Utilize artificial intelligence models, including Artificial Neural Networks (ANN) and Adaptive Neuro-Fuzzy Inference Systems (ANFIS), to support the development of MATLAB-based tools for advancing food safety and quality monitoring.
* Feasibility and Regulatory Assessment: Undertake studies to evaluate the economic viability, consumer acceptance, and regulatory compliance associated with the use of *Spirulina* extracts in food preservation. This line of research is essential for scaling up implementation in real-world food processing environments.

Study Limitations

This study investigating the antimicrobial activity of Spirulina platensis against total coliforms and Staphylococcus aureus in tilapia fillets is subject to several methodological limitations. The sample size was restricted to 25 fresh tilapia specimens, each divided into four portions, yielding 100 fillet samples. The antimicrobial effects were monitored over a relatively short duration—only at 1, 24, and 48 hours post-treatment. Additionally, only three concentrations of Spirulina extracts (0.5%, 1%, and 5%) were evaluated.

These constraints limit the generalizability of the findings. It is plausible that different results could be obtained with larger and more diverse sample sets, higher extract concentrations (e.g., above 5%), and extended observation periods. Future research should consider these variables to enhance the robustness and applicability of findings. Moreover, leveraging artificial intelligence tools across broader datasets and variables may further improve the predictive accuracy and utility of such studies. Integrating microbiological analysis, food safety protocols, and AI-driven modeling could foster innovative approaches to food preservation and help mitigate the risk of foodborne illness.

In this study, negative control groups were included to ensure that observed antimicrobial effects were attributable solely to the Spirulina treatments. However, the use of a positive control group—treated with a known antimicrobial agent—provided a comparative benchmark for evaluating Spirulina’s efficacy. Future studies are encouraged to include well-established positive controls such as nisin or organic acids to better contextualize the antimicrobial potential of Spirulina extracts.

Furthermore, Spirulina’s stability within different food matrices, including its pH sensitivity and potential sensory impacts (e.g., changes in color or flavor), requires further investigation to support its application in industrial food processing. We acknowledge this as a limitation and recommend that future research designs incorporate both positive and negative controls, along with sensory and physicochemical evaluations, for a more comprehensive assessment of Spirulina’s practical potential.

Data AvailabilityAll data generated and/or analyzed during this study are included in this published article and its supplementary information files.

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