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Microbial Quality of Salted Sun-Dried Fish and Antibacterial Efficacy of Green-Synthesized Copper Nanoparticles

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Abstract:

The present study investigated the microbial contamination of salted sun-dried sea fish and evaluated the antimicrobial properties of green-synthesized copper oxide nanoparticles (CuO NPs) produced using *Pongamia pinnata* root extract. Freshly collected samples of *Stolephorus indicus* were examined for bacterial load, followed by isolation and identification of contaminants using standard morphological and biochemical methods. Aqueous root extract of *P. pinnata* was used for the green synthesis of CuO NPs, which were subsequently characterized using UV–Visible spectroscopy and FTIR analysis. Four bacterial species *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* were identified from the fish samples. FTIR spectra revealed functional groups involved in nanoparticle reduction and stabilization, while UV–Visible analysis confirmed nanoparticle synthesis through characteristic SPR peaks. Antibacterial assays demonstrated strong inhibitory activity of the CuO NPs, with *Pseudomonas aeruginosa* exhibiting the highest susceptibility. Overall, the findings indicate that plant-mediated CuO NPs possess significant antimicrobial potential and may serve as effective natural agents for reducing bacterial contamination in dried fish products.

Keywords — Salted sun-dried fish, *Stolephorus indicus*, *Pongamia pinnata*, Green synthesis, Copper nanoparticles, FTIR, Antibacterial activity.

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I. INTRODUCTION

Salted sun-dried fish serves as an important dietary protein source across many regions of India, particularly in coastal and rural communities where it is valued for its low cost, extended shelf life, and high nutritional content [1]. Despite its popularity, the traditional methods used for salting, drying, and storing fish are often carried out in open environments without adequate sanitary control. These practices expose the fish to dust, insects, and contaminated surfaces, increasing the likelihood of microbial colonization. Numerous studies have reported that dried fish can harbour numerous pathogenic bacteria, such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, are known to cause foodborne diseases [2]. Fungal contaminants, particularly *Aspergillus* species capable of producing harmful mycotoxins, further elevate the risk associated with consumption of improperly processed fish.

Drug-resistant human pathogenic microorganisms have emerged largely due to the indiscriminate and prolonged use of commercial antimicrobial drugs in the treatment of infectious diseases. This growing challenge has compelled scientists to explore novel antimicrobial agents from diverse natural and alternative sources [3]. Concurrently, the emergence of antimicrobial resistance and increasing concerns over chemical preservatives have intensified the search for safer and more sustainable antimicrobial strategies [4]. Nanotechnology has drawn significant interest in this context, especially the utilization of metal-based nanoparticles as potent antimicrobial agents [5]. Among these, copper nanoparticles (CuONP) are widely recognized for their strong antimicrobial potency, economic feasibility, and structural stability. The shift toward green synthesis of nanoparticles represents a major advancement, as it employs plant-derived phytochemicals such as flavonoids, terpenoids, phenols, and alkaloids as organic reducing and stabilizing agents, doing away with the need for hazardous chemicals [6].

Pongamia pinnata, a medicinally important plant known for its rich phytochemical composition, has demonstrated considerable potential as a biological resource for nanoparticle synthesis [7]. Its root extracts contain bioactive compounds capable of reducing metal ions and stabilizing nanoparticles, making it an effective candidate for eco-friendly CuONP production [8]. The present study was undertaken to address the dual challenge of enhancing the microbiological safety of traditionally processed dried fish and identifying environmentally sustainable antimicrobial alternatives. By examining the extent of microbial contamination in salted sun-dried fish and exploring the potential of plant-derived copper nanoparticles as natural antimicrobial agents, this work aims to bridge gaps in current food safety practices. The study integrates microbial assessment with green nanotechnology, focusing on the synthesis of copper nanoparticles using *Pongamia pinnata* root extract and their subsequent characterization and evaluation against fish-associated pathogens. Through this comprehensive approach, the research contributes to the development of safer preservation methods and promotes the use of eco-friendly nanomaterials in food safety applications.

2. METHODOLOGY

2.1. The Collection of Samples

Saltwater-dried Fish samples of *Stolephorus indicus*was collected from a dry fish market located in the District of Perambalur, Tamil Nadu, India. Samples were placed into sterile containers and taken to the laboratory for the purpose of Performing Microbiological Analyses.

2.2. Isolation of Bacteria

Bacteria were isolated from salted sun-dried fish by homogenizing one gram of each sample in ten mL of sterile distilled water, followed by serial dilution up to 10^{-6}. From the 10^{-4}, 10^{-5}, and 10^{-6} dilutions, one mL aliquots were aseptically transferred into sterile Petri plates, after which molten Nutrient Agar was poured and gently mixed to ensure even distribution of microorganisms. The plates were incubated at 37°C for 24 hours, and the resulting colonies were enumerated to determine the bacterial load. Bacterial identification was carried out based on morphological, cultural, and biochemical characteristics following the standard protocols [9].

2.3 Preparation of *Pongamia pinnata* Root Extract

Fresh roots of *Pongamia pinnata* were collected from the local agricultural fields and immediately washed thoroughly under running tap water to remove the adhering soil, dust, and debris. The roots were further washed with distilled water and shade-dried for 7–15 days to prevent the deterioration of any heat-sensitive phytochemicals [10]. The roots were stored in sealed containers after being completely dried [11] and processed into a fine powder using a sterile mechanical grinder [12]. 50 g of the powdered root material and 400 mL of deionized water were combined for the aqueous extraction in a 500 mL conical flask that was securely covered with aluminum foil to keep out light. Additionally, the mixture was shaken mechanically for 90 minutes to help release the bioactive compounds, and then it was mildly heated to 50°C for one hour with a magnetic stirrer to help with the extraction process. Subsequent to heating, the mixture was left to cool down gradually followed by overnight incubation at room temperature for maximum diffusions of various phytochemicals into the solvent. This extract was filtered using Whatman No. 1 filter paper to get a particulate-free clear aqueous solution. The filtrate thus obtained was cooled and stored at 4°C until further use for nanoparticle synthesis.

2.4 Green Synthesis of Copper Nanoparticles

A 0.2 Molar solution of $CuSO_4$ sulphate solution was prepared freshly using analytical grade copper sulphate and deionized water and stored in amber-colored bottles to avoid photodegradation. To prepare copper nanoparticles, 0.1L of the *Pongamia pinnata* root extract was added drop wise in a slow manner to 0.4L of the $CuSO_4$ solution under continuous stirring using a magnetic stirrer to ensure proper mixing and controlled reduction of the copper ions [13]. The reaction mixture was allowed to incubate at room temperature for 24 h, during which a distinguishable colour change from blue to light brown was observed, indicating the reduction of Cu^{2+} ions followed by the formation of copper nanoparticles. At the end of the incubation period, the suspension was subjected to centrifugation at 10,000 rpm for 15 min to pelletize the synthesized nanoparticles. The thus obtained pellet was washed repeatedly with deionized water followed by ethanol in order to remove unreacted biomolecules and other impurities. The purified nanoparticles were dried in the open air at room temperature and kept for subsequent characterization by FTIR and UV–Vis spectroscopy.

2.5 Characterization of Copper Nanoparticles

The synthesized copper nanoparticles were subjected to standard analytical techniques for identifying the optical and functional properties of the materials. UV–Visible spectroscopy was carried out using a double-beam spectrophotometer, and the absorbance was recorded within the range of 200–500 nm to monitor the surface plasmon resonance behaviour typical of copper nanoparticles [14]. The nanoparticle functional groups were identified by performing Fourier Transform Infrared (FTIR) spectroscopy on a PerkinElmer spectrophotometer. Dried samples of nanoparticles were scanned over the mid-infrared region to identify the chemical groups in the plant extract participating in nanoparticle formation and stabilization. These analytical procedures enabled the assessment of biomolecules associated with the green synthesis and capping of copper nanoparticles.

2.6 Antimicrobial Activity Assay

The in vitro antimicrobial efficacy of the green-synthesized CuONPs was evaluated using the standard disc diffusion method [7]. For the antibacterial assay, common bacterial pathogens were isolated, identified by conventional morphological methods, microscopic and biochemical tests [15]. They were maintained as pure cultures in the department of bacteriology laboratory, Srinivasan College of Arts and Science, Perambalur. The pure cultures of actively growing of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were swabbed uniformly onto sterile Mueller–Hinton agar plates with the aid of sterile cotton swabs to obtain an even lawn. Sterile filter paper discs impregnated with the CuONPs were aseptically placed on the surface of the inoculated plates, followed by incubation at 37°C for 24 hours. The plates were examined after incubation, and the diameter of the zones of growth inhibition around the discs was measured in millimeters as an indicator of the antimicrobial potential of the copper nanoparticles. The most CuO nanoparticle sensitive bacterial isolate was identified by 16S rRNA sequencing. Genomic DNA was extracted, the 16S rRNA gene was PCR-amplified using universal primers 27F and 1392R under standard PCR conditions, and amplicons were sequenced bidirectionally. Sequences were analyzed using BLAST and phylogenetic analysis in MEGA X.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacteria from Salted Sun-Dried Fish

Microbiological analysis of the salted sun-dried fish samples revealed a high level of contamination, with *Stolephorus indicus* containing 210×10^6 CFU/g. Such elevated microbial loads clearly indicate unhygienic handling, exposure to open environments during drying, and poor storage conditions. Similar contamination levels have been reported in sun-dried fish processed under open and uncontrolled conditions, confirming the vulnerability of traditional drying methods to microbial proliferation [16]. Morphological and biochemical characterization confirmed four major bacterial isolates: *Staphylococcus aureus*, *Bacillus* *subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which are commonly associated with seafood and known for their salt-tolerance capabilities [17].

The presence of *E. coli* and *P. aeruginosa* is particularly concerning because both are widely recognized indicators of poor hygiene, contaminated water, and post-processing handling issues [18]. As *E. coli* is typically of fecal origin, its presence suggests contamination during cleaning or drying. Similar findings have been documented in dried fish processed without proper sanitation, raising significant public health concerns [19]. Furthermore, *S. aureus* a frequent contaminant in artisanal dried fish is capable of producing heat-stable enterotoxins, thereby posing a risk even after cooking. Its contamination is often attributed to human handling, inadequate personal hygiene, and airborne exposure during the drying process [20].

Overall, the microbial profile observed in the present study highlights the limitations of traditional sun-drying practices. These findings underscore the necessity of improving fish-drying protocols through hygienic platforms, solar-assisted drying units, controlled-environment chambers, and strict sanitation standards during handling and storage. Adoption of such improved systems would significantly reduce microbial contamination and enhance the safety of dried fish products.

3.2 Characterization of Green-Synthesized Copper Nanoparticles

3.2.1UV–Visible Spectroscopy

The UV–Visible analysis of the biosynthesized copper oxide nanoparticles revealed absorption peaks at 198.30 nm, 226.70 nm, and 392.55 nm. The strong peaks at 198.30 and 226.70 nm correspond to $\pi \rightarrow \pi^*$ transitions associated with aromatic compounds, flavonoids, and phenolic constituents of *Pongamia pinnata* root extract. Their presence indicates that phytochemicals bind to the nanoparticle surface and function as capping agents. Similar UV bands have been reported for CuO NPs synthesized using plant extracts such as *Moringa oleifera*, reinforcing the role of bioactive molecules in nanoparticle formation [21]. The peak at 392.55 nm represents the surface plasmon resonance (SPR) characteristic of copper oxide nanoparticles and falls within the typical SPR range (320–400 nm) for green-synthesized CuO NPs. This suggests the formation of small-sized nanoparticles. A slight blue shift further indicates the presence of smaller or partially oxidized copper species—an expected feature of plant-mediated synthesis where natural oxidation occurs during nanoparticle formation [22]. Collectively, the UV–Visible results confirm successful fabrication of CuO NPs and highlight the characteristic optical behavior influenced by Pongamia-derived phytochemicals.

3.2.2 FTIR Analysis

FTIR spectra of the synthesized CuO NPs displayed pronounced O–H stretching peaks at 3448 cm^{-1} and 3849 cm^{-1}, confirming the involvement of hydroxyl-rich phytochemicals—such as polyphenols, tannins, and flavonoids—in nanoparticle reduction and stabilization. The peak at 1644 cm^{-1} corresponds to C=C stretching of aromatic rings, indicating adsorption of bio-organic compounds onto the nanoparticle surface. These functional groups have been commonly reported in other Pongamia-mediated nanomaterials, reflecting the strong affinity of plant metabolites for metal ions during green synthesis [23]. The distinct absorption band at 577 cm^{-1} corresponds to Cu–O stretching vibrations, confirming the formation of copper oxide phases. CuO formation is typical in aqueous plant-mediated synthesis due to natural oxidation of reduced copper during nanoparticle development. Similar Cu–O vibrational peaks were reported in *Pongamia* leaf- and flower-derived CuO nanoparticles, supporting the metallophilic role of bioactive compounds in forming stable nanostructures [24]. Overall, the FTIR results verify that *P. pinnata* root extract serves both as a reducing and stabilizing agent, enabling environmentally friendly synthesis of stable CuO nanoparticles.

The FTIR results overall confirm that biomolecules present in *Pongamia pinnata* root extract act as dual functional agents—both reducing and capping the nanoparticles. This biological synthesis pathway is environmentally benign and relies on natural antioxidants inherent in the plant. These features not only ensure stable nanoscale formation but also enhance potential antimicrobial or catalytic properties due to the organic corona on the particle surface.

3.3 Antimicrobial Activity of Copper Nanoparticles

Disc diffusion method is simple, reliable, and official method used for routine antimicrobial susceptibility testing [25]. Although other methods such as agar plug, cross-streak [26], and well diffusion assays are also employed to assess antimicrobial activity, the disc diffusion method remains the most commonly accepted and officially recommended approach due to its reproducibility and ease of interpretation. The green-synthesized CuO NPs exhibited strong antibacterial activity against all tested fish pathogens. The inhibition zones recorded were 28 mm for *Pseudomonas aeruginosa* , *Bacillus subtilis* (23) mm *and Staphylococcus aureus* (21mm) and 18 mm for *E.coli*.

Using 16S rRNA gene sequencing, the bacterial isolate with the highest sensitivity to CuO nanoparticles was identified molecularly. A 1,417 bp partial 16S rRNA gene sequence was obtained using PCR amplification, examined, and added to the GenBank database with the accession number PP732494. A high level of sequence similarity with GenBank reference sequences was found by BLAST analysis, suggesting a close relationship to *Pseudomonas aeruginosa.* This affiliation was further supported by phylogenetic analysis using the Neighbor-Joining method, which showed that the isolate (VPJ1) grouped with several reference strains with significant bootstrap support inside the *P. aeruginosa* clade (Fig. 5). After pairwise removal of ambiguous sites, 1,425 nucleotide locations were used to build the phylogenetic tree, and the Maximum Composite Likelihood technique was used to determine evolutionary distances. Overall, the molecular identification corroborates the results obtained from conventional morphological and biochemical characterization, thereby confirming the taxonomic identity of the isolate as *Pseudomonas aeruginosa*.

The higher susceptibility of *Pseudomonas aeruginosa* is attributed to its porous layer, which facilitates easier penetration of metal ions and oxidative species generated by CuO NPs. These findings align with established antimicrobial mechanisms of copper nanoparticles, which include cell membrane disruption, oxidative stress induction via reactive oxygen species (ROS), protein denaturation, and interference with DNA replication [27]. The antimicrobial activity observed mirrors the results from recent studies using *Pongamia*-mediated CuO NPs, where similar inhibition zones were reported for *E. coli* and *S. aureus* [24]. Saravanakkumar et al. [8] also reported potent antibacterial efficacy of Ag-doped CuO NPs synthesized from *Pongamia* flowers, with inhibition zones ranging from 16–28 mm. Furthermore, Raveendran et al. [28] demonstrated comparable antibacterial activity in CuO NPs synthesized using *P. pinnata* stem extracts, attributing the strong bioactivity to the small particle size and the presence of phenolic capping agents that enhance penetration into bacterial membranes. Notably, the pronounced antibacterial activity of the green-synthesized CuO NPs against *P. aeruginosa* in this study aligns well with the findings of Wang et al. [29], who reported significant growth inhibition at concentrations as low as 8µg/mL and enhanced bactericidal effects at 16 µg/mL, along with effective suppression of *P. aeruginosa* induced lung infections at an in vivo dose of 200 µg/kg. Collectively, these results suggest that plant-mediated CuO nanoparticles possess broad-spectrum antimicrobial activity and hold strong potential for applications in food preservation, biomedical textiles, wound management, and the control of microbial contamination in dried seafood products.

3.4.Conclusion

The present study demonstrates that salted sun-dried fish harbour considerable bacterial contamination, including important foodborne pathogens such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*. These findings highlight the need for improved hygiene and controlled-environment drying systems to reduce microbial risks. The study successfully synthesized copper oxide nanoparticles using *Pongamia pinnata* root extract, with UV–Visible and FTIR analyses confirming the role of phytochemicals in nanoparticle formation and stabilization. The biosynthesized CuO NPs exhibited potent antibacterial activity, particularly against *P. aeruginosa*, demonstrating their potential as eco-friendly antimicrobial agents. Their application in dried fish processing and packaging could significantly enhance food safety and extend shelf life. Future research should focus on toxicity assessment, mechanistic studies, and development of CuO SSSNP based antimicrobial packaging systems suitable for industrial use.

Figure 1: Salted sun dried fish *Stolephorus indicus*

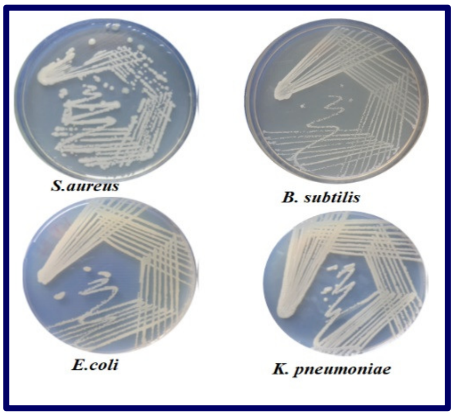

Figure 2 : Pure culture of bacterial isolates

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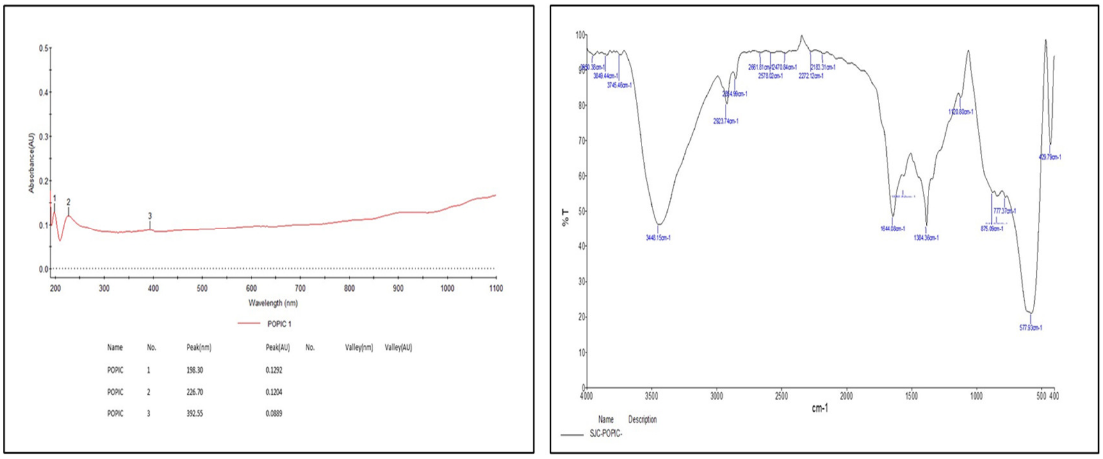

Figure 3:UV–Visible and FTIR spectra showing the optical properties and functional groups involved in the green synthesis and stabilization of CuO nanoparticles using *Pongamia pinnata* root extract.

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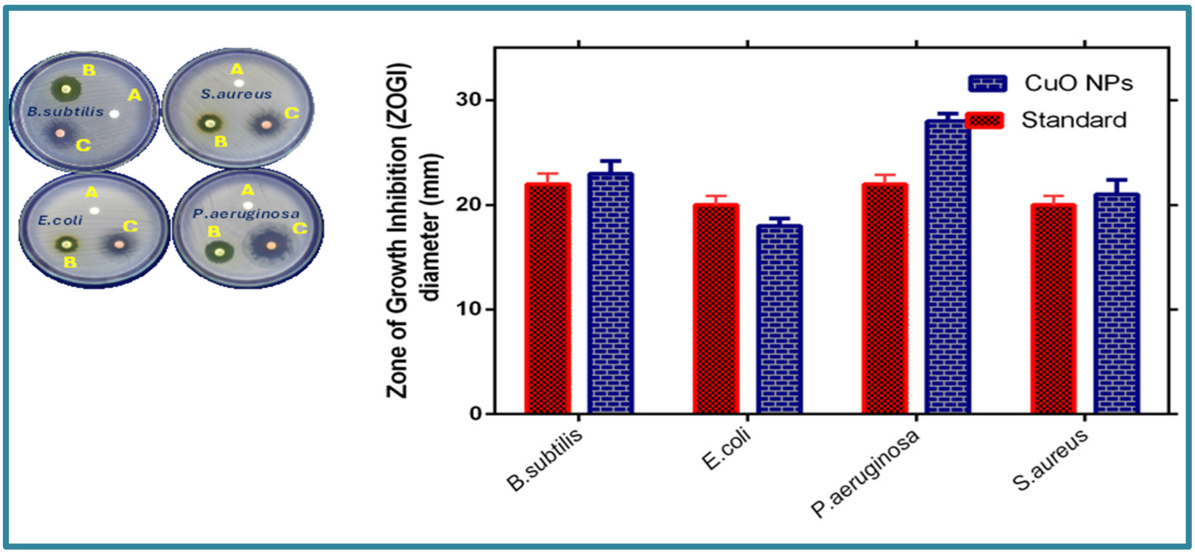

Figure 4. Antibacterial activity of CuONPs against bacterial isolates. A. Disc diffusion method (A-Control, B-Standard (Nitrofurantonin (100mcg) , C- CuONPs). B. Bar diagram illustrating the zone of growth inhibition (mm) exhibited by green-synthesized CuO nanoparticles against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in comparison with a standard antibiotic.

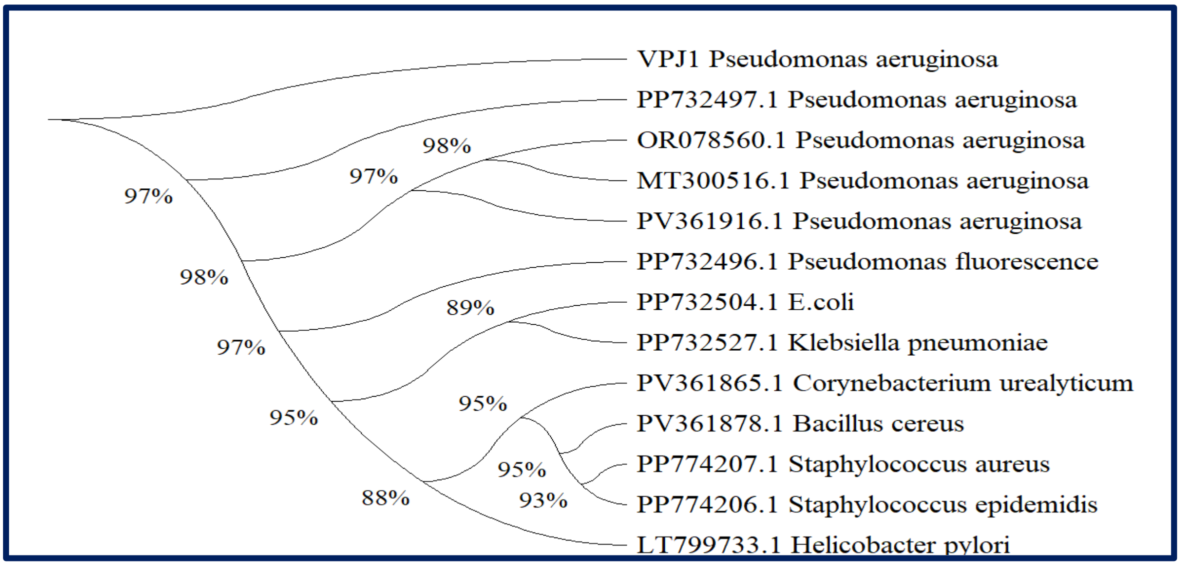

Figure 5. Phylogenetic analysis of bacterial isolates based on 16S rRNA gene sequences.
Evolutionary relationships among 13 taxa were inferred using the Neighbor-Joining method. Evolutionary distances were calculated using the Maximum Composite Likelihood approach and are expressed as the number of base substitutions per site. Bootstrap support values (%) are shown at the nodes. The analysis included 1,425 nucleotide positions after pairwise deletion of ambiguous sites. The optimal tree (sum of branch length = 0.828) demonstrates clustering of isolate VPJ1 with *Pseudomonas aeruginosa*. Phylogenetic analyses were performed using MEGA12.

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