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Assessment of *In vitro* Potential of Turmeric Crude Extract Against *Klebsiella pneumoniae* on Smoked Nile Tilapia (*Oreochromis niloticus*)

Louie Jay T. Biquio [a], Clutha C. Pongan [a], Renz Ivan M. Sabado [a], Jayson V. Bernabe [a], Joy C. Gonzales [a], Laika Camille S. Sapigao [a], Kelvin Ronald DL. Nudo [a], Greg Valen M. Dela Cruz [a], Charish A. Cabanisas [a], Melissa D. Pasion [a], Jonalyn M. Alonzo [a], Richelle Mae B. Butial [a], Monica M. Edralin [a], Hanz Joshua M. Guillermo [a], Joanna S. Policarpio [a], Carl Jayson M. Sirot [a], Princess Allen O. Valdez [a], Noel Loie Zamora [a] and Alvin T. Reyes [a*]

[a] Department of Aquatic Post-Harvest, College of Fisheries, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: Email: alvinreyes@clsu.edu.ph;*

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Original Research Article

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ABSTRACT

This study investigated the potential of turmeric (*Curcuma longa*) crude extract to inhibit *Klebsiella pneumoniae* in smoked Nile tilapia. Initial tests showed that 5.0 mL of a 0.5 g/mL extract concentration exhibited significant antibacterial activity, resulting in a marked reduction in bacterial growth compared to other treatments. To confirm its effectiveness, the optimal extract from the preliminary test was applied to smoked fish experimentally infected with *Klebsiella pneumoniae*. The results indicated that the optimal extract had fewer bacterial count up to Day 5 as compared to control, but showed decreased efficacy by Day 5. Further research is needed to enhance its effectiveness and address limitations, promoting sustainable food safety practices in the aquatic food industry.

Keywords: Food safety; Klebsiella pneumoniae; smoked fish; turmeric.

1. INTRODUCTION

Food safety refers to the proper food handling procedures applied during food preparation, processing, storage, and distribution of the products. Food safety is crucial for maintaining and promoting the health of those who consume it (Liivat, 2024). Access to sufficient amounts of safe and nutritious food is key to sustaining life and promoting good health. Unsafe food containing harmful bacteria, viruses, parasites or chemical substances causes more than 200 diseases, ranging from diarrhea to cancer (World Health Organization, 2022). Food safety is a significant concern worldwide, as contaminated food can lead to various health issues, including foodborne illnesses. The production and consumption of nutritious and safe food is central to all food systems, including the aquatic food industry. The latter has undergone tremendous expansion, reaching a record of 214 million tons in 2020 (Food and Agriculture Organization, 2022). Small-scale fisheries contribute approximately half of global fish catches (Food and Agriculture Organization, 2024).

Foods are categorized into less perishable, moderately perishable, and highly perishable based on their stability (Singh et al., 2015). Seafood is highly perishable due to its high moisture content and nutrients. Ambient temperature can significantly affect the stability of these foods, with moderately perishable foods like fruits and vegetables being more tolerant than highly perishable ones like seafood, and non-perishable foods being least impacted (Singh et al., 2015). Food spoilage can be defined as “any sensory change (tactile, visual, olfactory or flavor)” that the consumer considers to be unacceptable (Rawat, 2015). Spoilage may occur at any stage along the food chain. Spoilage may arise from insect damage, physical damage, and indigenous enzyme activity in the animal or plant tissue or by microbial infections (Catteau et al., 2017; Daily et al., 2016). Most natural foods have a limited shelf life. Perishable foods such as fish, meat and bread have a short life span. Despite of the proven efficiency of these chemical preservative in prevention and outbreak control of food poisoning diseases, their repeated applications have resulted in the accumulation of chemical residues in the food and feed chain, acquisition of microbial resistance to the applied chemicals, and the unpleasant side effects of these chemicals on human health (Bialonska et al., 2010).

Smoking is a preservation method that exposes fish to smoke from the incomplete combustion of wood, either directly or indirectly. This process enhances flavor, texture, and shelf life by reducing moisture and microbial load while preventing enzymatic and chemical deterioration, making smoked fish more appealing and long lasting (Sowumi, 2007). Despite its preservative benefits, smoked fish can still be susceptible to microbial contamination due to various factors, including water pollution, poor hygienic conditions during processing, improper handling, and inadequate storage. Additionally, failure to follow Good Manufacturing Practices (GMP) can lead to the presence of harmful microorganisms such as coliforms, *Escherichia coli*, and molds, compromising both food safety and nutritional value (Myrna et al., 2024).

Enterobacteriaceae are a large, diverse heterogeneous group of rod-shaped Gram-negative bacilli that survive under aerobic conditions and normally inhabit the intestines of man and animals; some are motile while some others are not. The family includes many genera, some of which are part of the normal flora and incidentally cause diseases especially when given the opportunity. They are non-spore forming and some have capsules while others do not. Enterobacteriaceae can be considered common waterborne fish infections, frequently found in the tissues of seemingly healthy fish and the gastrointestinal tracts of humans and animals (Speranza et al., 2021). The presence of fecal coliforms in fish serves as an indicator of the contamination level in their living environment (Centers for Disease Control and Prevention, 2013).

Numerous studies have looked into the antibacterial qualities of plants all over the world, and many of these studies have employed the antimicrobial qualities of plants as medicinal alternatives. Researchers are investigating the use of plant extract in the treatment of infections as a means of finding a viable and cost-effective alternative in the face of rising antimicrobial medication resistance. By employing natural antimicrobial agents extract, the food industry can develop eco-friendly and effective alternatives to synthetic preservatives, contributing to sustainable food production practices and improving overall food safety (Chassagne et al., 2021).

2. MATERIALS AND METHODS

2.1 Collection and Drying of Turmeric

Matured turmeric, around 300 g was collected within the vicinity of Science City of Muñoz, Nueva Ecija, Philippines. The turmeric was sun-dried for 1 hour and oven-dried for 4 hours at 70 °C to obtain the desirable moisture content. The dried turmeric was pulverized using a blender (Farid et al., 2016)].

2.2 Crude Extraction of Powdered Turmeric

To come up with 0.50 g/mL concentration, the dried turmeric was boiled in distilled water for 15 minutes following the 1 g:2 mL ratio (150 g rhizomes:300 mL distilled water). The decoction was filtered using a filter paper. The filtrate was transferred in test tubes and stored in a chiller until use (Reyes et al., 2019).

2.3 Acquisition and Preparation of *Klebsiella pneumoniae*

Pure culture of *K. pneumoniae* strain DSM 30104 was obtained from the study "Antibiotic Resistance of Esculin-hydrolyzing Bacteria Isolated from Tilapia Farms with Different Water Source in Pampanga, Philippines." The identity of the isolate was confirmed by 16S rRNA sequencing. The bacterium was sub-cultured in Mueller Hinton Agar (MHA) plate. About 2 to 3 colonies of the bacterium were suspended in 20 mL Mueller Hinton Broth (MHB). After 18 to 24 hours of incubation in room temperature, the bacterial suspension was adjusted to 0.5 McFarland standard (Baltazar et al., 2020).

2.4 Assessment of *In Vitro* Potential of Turmeric Crude Extract Against *Klebsiella pneumoniae*

The disposable Petri plates were loaded with appropriate volume of melted MHA and prepared crude extract of turmeric (Table 1). Each MHA plate was pipetted with 0.1 mL of the adjusted *K. pneumoniae* suspension; the loaded suspension was spread on the surface of the MHA plates using sterilized L-rod. The plates in triplicate were incubated for 18 to 24 hours under room temperature.

After the incubation period, the treatment with least bacterial growth was used in the next phase of experiment.

Table 1. The various treatments with their corresponding volume of melted Mueller Hinton Agar and turmeric crude extract

	Volume of Melted MHA (mL)	Volume of Crude Extract (mL)
Treatment 1 (Control)	20.0	0.0
Treatment 2	19.0	1.0
Treatment 3	18.0	2.0
Treatment 4	17.0	3.0
Treatment 5	16.0	4.0
Treatment 6	15.0	5.0

Nile tilapia was smoked following the procedures of the course AQPH 3305 (Post-Harvest Fisheries) of the College of Fisheries in Central Luzon State University. Six (6) pieces of tilapia, with an average weight of 200 g, were eviscerated through the gill opening. The fish was washed thoroughly in running water, soaked in 10% brine solution for 1 hour, and was drained afterwards. The fish was sun-dried for 30 to 40 minutes until the fish was “dry to touch”. The fish were arranged in trays and were smoked for 30 to 45 minutes or until golden brown. The fish was cooled before using it in the next experiment.

The adjusted bacterial suspension (around 20 mL) was sprayed evenly on one side of the smoked tilapia in all groups. The treatment presented in Table 1 that resulted in least bacterial growth was sprayed in group 2 after 20 minutes prior to the first spray. In this case, the volume of melted MHA was replaced by sterilized distilled water (Table 2). The smoked fish was stored in a closed chamber for a period of 5 days.

Table 2. Description of the groups of smoked tilapia

	Description
Group 1 (Control)	Sprayed with *K. pneumoniae* only
Group 2	Sprayed with *K. pneumoniae* and best treatment

Two series of 10-fold dilution using sterilized water as a diluent was prepared in four test tubes. In each group, 1 g of flesh was taken and serially diluted until the fourth tube. One hundred microliters (100 µL) of the diluted sample in test tubes 3 (10^{-3}) and 4 (10^{-4}) were spread on the surface of MHA plates in triplicates. The number of colonies in MHA plates were counted and the colony forming units per gram (CFU/g) was computed using the below formula (Gupta et al., 2015). The bacterial counting in smoked tilapia groups was done after 3 days and 5 days prior to the spraying of the bacterial suspension and best extract concentration.

$$CFU\ per\ mL = \frac{Average\ no.of\ colonies\ x\ dilution\ factor}{Vp}$$

$$CFU\ per\ mL = \frac{\Sigma C}{((1\ x\ n1)+(0.1\ x\ n2))(d\ x\ Vp)}$$

Where:
C = colony counts
n1 = no of plates in 1st dilution counted
n2 = no of plates in 2nd dilution counted
d = dilution from which 1st counts were obtained
Vp = volume plated

2.5 Statistical Analysis

The bacterial count was log transformed first before subjected to statistical analysis. A paired T-test was used to statistically compare the bacterial count in tilapia groups sprayed with bacterial suspension only and those sprayed with both bacterial suspension and best extract concentration.

3. RESULTS AND DISCUSSION

Treatments 1 to 5 had uncontrolled growth of *K. pneumoniae*, indicating that these turmeric extract concentrations were insufficient to inhibit the bacterium. On the other hand, T6 (5 mL of 0.5 g/mL concentration) showed fewer bacterial growths, which served as basis for its selection as the best treatment for further testing. The observed inhibition in this study suggests that turmeric extract, particularly at higher concentrations, holds promise as an alternative or complementary approach to conventional antibiotics in controlling foodborne pathogens, especially in settings where the use of synthetic chemicals is discouraged. This aligns with a growing body of literature that supports the antimicrobial properties of curcumin, the primary bioactive compound found in turmeric (Dai et al., 2022). Curcumin has been shown to exert antibacterial effects through mechanisms such as disruption of bacterial cell membranes, inhibition of bacterial enzymes, and modulation of gene expression related to pathogenicity. The compound curcumin has been found to have a dose-dependent effect on its antimicrobial properties; the higher concentrations of curcumin tend to lead to greater antibacterial activity. Turmeric extract contains other compounds with antibacterial properties, including steroids, flavonoids, terpenoids, alkaloids, and anthraquinones (Hussain et al., 2007). This aligns with the findings of Zaika and Kissinger (1981), who noted that herbal medicinal plants contain secondary metabolites that contribute to their effectiveness in treating various ailments.

The effectiveness of an extract depends on various factors, including its potency, which can vary due to different extraction methods and concentrations. Furthermore, there's often limited research directly comparing different extract potencies for specific conditions, making it difficult to determine a universally effective dosage (Aggarwal et al., 2007).

Table 3. Bacterial count in the flesh of smoked Nile tilapia after 3 and 5 days of experiment

Groups	Bacterial Count (CFU/g)	
	Day 3	Day 5
Group 1 (Control)	8.10×10^6	2.27×10^7
Group 2	$8.00 \times 10^{5*}$	2.10×10^6

**denotes significant difference at P <0.05 level*

Previous studies have also demonstrated the inhibitory effects of turmeric on various pathogens, including Gram-negative bacteria like *K. pneumoniae* (Hussain et al., 2022). Wada et al. (2021) reported that an aqueous turmeric extract demonstrated an 11 mm inhibition zone against *Staphylococcus aureus* and a 7 mm inhibition zone against *K. pneumoniae* at a concentration of 100 mg/mL. In the same study, the ethanol extract of turmeric produced an inhibition zone of 12 mm against *S. aureus*, 10 mm against *Escherichia coli*, and 7 mm against *K. pneumoniae* at the same concentration. Similarly, Sylvester et al., (2015) found that Java turmeric extract exhibited susceptibility to all resistant *K. pneumoniae*, with inhibition zones ranging from 8.67±0.58 mm to 10.00±0.00 mm. Ungphaiboon et al. (2005) determined the Minimum Inhibitory Concentration (MIC) of curcumin to be 16 µg/mL for *Bacillus subtilis* NCTC 10073 and 128 µg/mL for *S. aureus* ATCC 25923 but observed no activity against *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Anbari et al., (2021) found that the MIC values of curcumin nanoparticles ranged from 0.48 to 0.34 mg/mL, whereas pure curcumin exhibited an MIC of 0.56 mg/mL.

The second phase of experiment focused on the application of T6, previously identified as the most potent antibacterial treatment. This part of the study sought to validate its effectiveness in a real-world context, emphasizing its potential for enhancing the microbial safety of smoked fish products. The smoked fish in Group 2, that were sprayed with both the bacterium and the best crude extract concentration, yielded significant lower count of bacteria after 3 days (8.0×10^5 CFU/g) as compared to Group 1 (8.1×10^6 CFU/g) (P <0.05). Lower count of bacteria was also recorded in Group 2 after 5 days but not statistically significant (P >0.05). There was an increased bacterial count in both groups after 5 days of the experiment. The lesser bacterial count in Group 2 after 3 days suggests a notable antibacterial effect of the crude extract. However, this effect was short-lived, as the bacterial count increased after 5 days. This indicates that while the crude extract exhibited initial efficacy in suppressing *K. pneumoniae* growth, its potency was not sustained over time. These findings highlight the complex interplay between the application of plant-derived antibacterial agents and the dynamic microbial environment of food products. While the study demonstrates the potential of the crude extract in reducing bacterial counts, it also emphasizes the need for further research to understand and overcome the limitations of its efficacy. Hypothetically, several factors could contribute to the short-lived effect of the crude extract. The extract's chemical composition may degrade over time, reducing its antibacterial potency. Additionally, the bacterial population may develop resistance to the extract, leading to its decreased effectiveness. The environmental conditions of the smoked fish, such as temperature and humidity, could also influence the efficacy of the extract. Potential contributors to the decline in efficacy of the extract include the degradation of curcumin, turmeric's primary antibacterial compound, which is sensitive to environmental factors like light and temperature (Gupta et al., 2015). Other reasons for unsustained efficacy over time are interference from other components in the food matrix, or inadequate absorption and retention of the extract on the surface of the fish. Additionally, *K. pneumoniae* and other bacteria may exhibit adaptive responses to antimicrobial agents, and a fixed volume of 5 mL (0.50 g/mL concentration) may have been insufficient to sustain antimicrobial effects (Gupta et al., 2015).

In fish and fishery products, enterotoxins produced by *S. aureus* can cause gastroenteritis upon consumption. Turmeric's antibacterial activity, attributed to curcumin binding to bacterial cell walls and causing cell lysis, may help inhibit such effects (Moghadamtousi et al., 2014). Aulia et al. (2019) observed that 5% turmeric extract extended the shelf life of presto lalawak fish (*Barbodes balleroides*) to 5 days at room temperature, based on microbial counts, pH levels, and sensory evaluations. Korkmaz et al. (2019) found that turmeric-coated rainbow trout fillets showed the highest inhibition of microbial spoilage among samples treated with

turmeric. Turmeric coating reduced lipid oxidation, protein denaturation, and total volatile basic nitrogen (TVB-N) levels. Similarly, Pezeshk et al. (2011) reported that dipping vacuum-packaged rainbow trout (*Oncorhynchus mykiss*) in turmeric or shallot extract slowed chemical changes (TVB-N, PV, and TBA) and microbial growth, extending shelf life. Arulkumar et al. (2017) demonstrated that turmeric extract inhibited the growth of mesophilic, psychrophilic, *Pseudomonas* spp., and biogenic amine-forming bacteria in cuttlefish (*Sepia brevimana*) stored at 4°C, adding 3 days of shelf life compared to untreated samples. Jana and Chakraborti (2016) found that combining turmeric and salt minimized protein denaturation in fish for up to 15 days, a practice commonly used in households. Nahid et al. (2016) reported that turmeric and salt, followed by smoke-drying, shortened drying times, preserved nutritional quality, and extended the shelf life of fish. Turmeric and salt-treated sun-dried shoal (*Channa striatus*), taki (*Channa punctatus*), and tengra (*Mystus tengara*) displayed excellent sensory properties, reduced protein and fat degradation, and acceptable microbial loads (<105 CFU/g (Farid et al., 2016). Mosarrat et al. (2017) reported improved sensory, chemical, and nutritional quality in turmeric and salt-treated smoke-dried fish species such as *Gudusia chapra*, *Xenentodon cancila*, and *Macrognathus pancalus*. Handayani et al. (2018) highlighted the effectiveness of 3 to 6% turmeric combined with 2 to 4% tamarind in reducing microbial loads in yellow seasoned pindang fish. Chilek et al. (2017) found the best preservative effects with a combination of turmeric, salt, and sodium acetate in refrigerated tilapia. Finally, Minh et al. (2019) demonstrated that soaking snakehead (*Channa striata*) in 0.15% curcumin and 0.3% salt for 20 minutes, followed by drying at 40°C for 20 hours, resulted in favorable physicochemical, microbiological, and sensory attributes.

4. CONCLUSION

The study highlights the potential of *C. longa* (turmeric) crude extract as a natural antibacterial agent against *K. pneumoniae*, with significant efficacy observed at higher volume concentration (5.0 mL of 0.5 g/mL concentration). However, its application on smoked Nile tilapia demonstrated a lesser bacterial growth only during the initial stage (Day 3), with diminished effectiveness observed by Day 5, suggesting that factors such as bacterial adaptation, degradation of active compounds, or insufficient dosage may have influenced the outcomes.

These findings underscore the need for further research to optimize turmeric extract's stability, formulation, and application methods to enhance its antimicrobial potential for practical use in food preservation. With appropriate refinements, *C. longa* could serve as a valuable natural alternative for controlling bacterial contamination in food products, contributing to improved food safety and reduced reliance on synthetic antimicrobial agents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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