OPEN ACCESS Freely available online
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Original Article
Bangladesh Journal of Infectious Diseases
December 2025, Volume 12, Number 2, Page 260-266
ISSN (Online) 2411-670X
ISSN (Print) 2411-4820
NLM ID: 101761093
DOI: https://doi.org/10.3329/bjid.v12i2.85869

In Vitro Evaluation of the Antibacterial Activity of *Zingiber officinale* (Ginger) Against *Salmonella* Typhi

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Abstract

Background: Antimicrobial resistance in *Salmonella* Typhi poses a significant public health challenge, particularly in developing countries like Bangladesh. Plant-based therapies are potential alternative antimicrobial agents due to their bioactive compounds. **Objective:** This study was aimed to evaluate the in vitro antibacterial activity of crude ginger extract (CGE) and ethanolic ginger extract (EGE) against *Salmonella* Typhi and compare their efficacy with the standard antibiotic Amikacin. **Methodology:** This experimental study was conducted at the Department of Pharmacology and Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Bangladesh, from July 2016 to June 2017. Crude and ethanolic extracts of ginger were prepared from fresh rhizomes. Antibacterial activity was assessed using nutrient agar incorporation for crude ginger extract (CGE) and disc diffusion assays for ethanolic ginger extract (EGE). Minimum inhibitory concentrations (MICs) were determined using broth dilution techniques. **Results:** CGE inhibited *Salmonella* Typhi growth at concentrations ≥50%, with complete inhibition at 100% concentration. EGE showed strong, dose-dependent antibacterial activity, with inhibition zones measuring 14 mm, 30 mm, and 33 mm at 25, 50, and 100 µg/10 µl, respectively. The MIC of EGE was 500 µg/ml, while Amikacin exhibited a significantly lower MIC of 1.5 µg/ml, confirming its higher potency. Subculture studies demonstrated bactericidal effects for CGE at 100%, EGE at 500 µg/ml, and Amikacin at 1.5 µg/ml. The study highlights that EGE was more effective than CGE, but less potent than the standard antibiotic, supporting its potential use as a complementary antimicrobial agent. **Conclusion:** Both crude and ethanolic ginger extracts demonstrated antibacterial activity against *Salmonella* Typhi, with ethanolic extract showing stronger, dose-dependent effects. While Amikacin was more potent, ginger extracts may serve as potential natural antimicrobial agents. [*Bangladesh Journal of Infectious Diseases, December 2025;12(2):260-266*]

Keywords: *Salmonella* Typhi; ginger extract; antibacterial activity; MIC; natural antimicrobial

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Introduction

Antimicrobial resistance has emerged as a critical global health challenge, especially in developing countries where infectious diseases remain a leading cause of morbidity and mortality. The increasing prevalence of multidrug-resistant pathogens, including *Salmonella* Typhi, has reduced the effectiveness of conventional antibiotics, creating substantial public health challenges[1]. *Salmonella* Typhi, the causative agent of typhoid fever, is particularly prevalent in South Asia, including Bangladesh, and parts of Africa, where poor sanitation, contaminated water, and limited healthcare access exacerbate disease transmission. In Bangladesh, typhoid fever continues to impose a significant health burden, with frequent outbreaks and increasing reports of resistance to first-line antibiotics such as ampicillin, chloramphenicol, and fluoroquinolones, as well as reduced susceptibility to third-generation cephalosporins[2]. This situation underscores the urgent need for safe, effective, and affordable alternative therapeutic strategies.

Medicinal plants offer a promising source of bioactive compounds with antimicrobial, antiviral, antioxidant, and anti-inflammatory properties. Plant-based therapies are increasingly recognized for being cost-effective, widely available, culturally accepted, and generally associated with fewer side effects compared to conventional drugs. In Bangladesh, more than 80.0% of the population relies on traditional herbal remedies for primary or complementary healthcare, particularly in rural areas where modern medical facilities are limited[3].

Among these plants, ginger (*Zingiber officinale*) is widely valued for both culinary and therapeutic uses. The rhizome contains numerous bioactive compounds, including gingerols, shogaols, zingerone, paradols, and sesquiterpenes, which have demonstrated antioxidant, anti-inflammatory, antipyretic, analgesic, and antimicrobial activities. Ginger's antimicrobial effects are mediated through mechanisms such as disruption of bacterial cell membranes, inhibition of key enzymatic processes, and suppression of microbial replication, making it a promising candidate against drug-resistant bacterial infections[4].

Previous studies have shown that ginger exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, and various *Salmonella* species[5]. Its extensive use in Bangladeshi traditional medicine, as well as in South Asian, Chinese, Middle Eastern, and African medicine, underscores its long-standing role in managing gastrointestinal infections and other microbial diseases[6,7]. However, few studies have specifically evaluated the comparative antibacterial efficacy of different ginger preparations, such as crude paste versus ethanolic extracts, against *Salmonella* Typhi. Given the increasing burden of multidrug-resistant *Salmonella* Typhi in Bangladesh and the need for alternative antimicrobial agents, this study aims to investigate the in vitro antibacterial activity of crude ginger paste and ethanolic ginger extract against *Salmonella* Typhi.

Methodology

Study Design and Setting: This experimental study was conducted in the Department of Pharmacology and Therapeutics in collaboration with the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh, between July 2016 and June 2017. The study aimed to evaluate the antibacterial activity of ginger (*Zingiber officinale*) against *Salmonella* Typhi using both crude and ethanolic extracts.

Materials: Fresh ginger rhizomes were purchased from the local market in Mymensingh, Bangladesh, and used for preparing crude and ethanolic extracts. Amikacin, an aminoglycoside antibiotic in injectable form, was procured from local pharmaceutical suppliers. The bacterial strain used for this study, *Salmonella* Typhi ATCC 14028, was obtained from the Department of Microbiology, Mymensingh Medical College, and maintained under standard laboratory conditions. Nutrient agar (NA) and nutrient broth (NB) were prepared following standard microbiological protocols, and all glassware and instruments were sterilized by autoclaving or hot air oven as appropriate.

Preparation of Crude Ginger Extract: Fresh ginger (1000 g) was washed thoroughly with distilled water, followed by rinsing with 95.0% ethanol to remove surface contaminants. The rhizomes were peeled, cut into small pieces, and homogenized using a sterile mortar and pestle. The homogenized material was then filtered through a double layer of sterile fine mesh cloth to obtain the crude ginger extract (CGE), which was considered as 100.0% CGE. The CGE was incorporated into nutrient agar to prepare different concentrations ranging from 5.0% to 100.0% (v/v). The required volume of CGE was mixed with distilled water and 2.8 g of nutrient agar to make a final volume of 100 ml per set. The mixtures were sterilized by autoclaving and poured into pre-sterilized Petri dishes. Control plates were prepared without CGE for comparison.

Inoculation and Incubation for CGE Assay: A bacterial suspension of *Salmonella* Typhi was prepared by selecting 3 to 5 similar colonies from 18–24 hour old nutrient agar plates and suspending them in sterile normal saline. The turbidity was adjusted to match 0.5 McFarland standard, corresponding to approximately 1.5 × 10^8 CFU/ml. The nutrient agar plates containing varying concentrations of CGE were inoculated by streaking with a sterile cotton swab dipped in the bacterial suspension. The plates were incubated at 37°C for 24 hours, after which bacterial growth was visually assessed. The absence of visible growth indicated inhibition of bacterial growth by the CGE.

Preparation of Ethanolic Ginger Extract: For the preparation of ethanolic ginger extract (EGE), peeled and chopped ginger rhizomes were dried in a shaded room for three days and then ground into a fine powder. Ten grams of powdered ginger was mixed with 200 ml of 95% ethanol and left at room temperature for 24 hours. The mixture was filtered through gauze and centrifuged at 3000 rpm for 10 minutes to remove particulate matter. The filtrate was further clarified using filter paper and then dried at 40°C in a hot water bath. The dried extract was stored at 4°C until use. For disc diffusion assays, a parent solution was prepared by dissolving 1 g of the dried ethanolic extract in 10 ml ethanol. From this stock solution, different concentrations were prepared by dilution with ethanol to achieve desired doses for antibacterial testing.

Disc Diffusion Assay for Ethanolic Ginger Extract: The antibacterial activity of EGE against *Salmonella* Typhi was evaluated using the disc diffusion method. Nutrient agar plates were inoculated with bacterial suspension standardized to 0.5 McFarland. Sterile blank discs were placed on the agar surface, and aliquots of EGE at different concentrations were applied onto the discs. Plates were left at room temperature for 5–10 minutes to allow diffusion and then incubated at 37°C for 24 hours. Zones of inhibition around the discs were measured in millimeters to determine antibacterial activity.

Determination of Minimum Inhibitory Concentration (MIC) of EGE: The MIC of EGE was determined using a broth dilution technique. Stock EGE solution was prepared by dissolving 1 g of powdered ginger extract in 10 ml ethanol. A further dilution (1:100) was made to obtain the working solution. Different concentrations of EGE were prepared by mixing measured volumes of working solution with nutrient broth to achieve final concentrations ranging from 100 µg/ml to 900 µg/ml. Each test tube containing the EGE dilutions was inoculated with 20 µl of standardized *Salmonella* Typhi suspension. Control tubes included broth with inoculum without extract and broth only without inoculum. The inoculated tubes were incubated at 37°C for 18–24 hours, and bacterial growth was assessed visually. The lowest concentration of EGE that prevented visible bacterial growth was recorded as the MIC.

Determination of MIC of Amikacin: Amikacin stock solution was prepared by dissolving 500 mg of injectable Amikacin in 500 ml sterile distilled water to achieve a concentration of 1 mg/ml. A 1:100 dilution of this stock yielded a solution containing 10 µg/ml, which was used to prepare serial dilutions in nutrient broth for MIC determination. Each tube was inoculated with 20 µl of *Salmonella* Typhi suspension and incubated at 37^0 C for 18 to 24 hours. The MIC was recorded as the lowest concentration of Amikacin that inhibited visible bacterial growth.

Subculture Studies: To confirm the inhibitory effects observed in previous experiments, subcultures were performed from CGE, EGE, and Amikacin preparations. Samples from tubes or plates showing inhibition and the last two sets showing bacterial growth were streaked onto nutrient agar plates without any extract or antibiotic. The plates were incubated at 37^0 C for 18–24 hours, and bacterial growth was examined to validate the results of MIC and antibacterial activity testing.

Statistical Analysis: Data were compiled and analyzed using Microsoft Excel. Antibacterial activity was assessed based on bacterial growth patterns, zones of inhibition, and minimum inhibitory concentration (MIC) values. Disc diffusion assays were performed in triplicate, and mean inhibition zones were recorded in millimeters (mm).

Ethical Consideration: This study was an in vitro experimental laboratory study involving bacterial isolates only and did not include human participants or animals. Therefore, formal informed consent was not required. All experimental procedures were conducted following standard microbiological laboratory safety and institutional research guidelines of Mymensingh Medical College, Bangladesh.

Results

The incorporation of crude ginger extract (CGE) into nutrient agar inhibits the growth of *Salmonella* Typhi ATCC 14028 in a concentration-dependent manner. No inhibition was observed at concentrations from 5.0% to 40.0%. Growth inhibition began at 50.0% CGE, and complete inhibition was achieved at 100.0% CGE. The control plate without CGE exhibited heavy bacterial growth, confirming the viability of the inoculum and the effectiveness of CGE at higher concentrations (Table 1).

Table 1: Inhibitory Effect of Crude Ginger Extract (CGE) on the Growth of *Salmonella* Typhi ATCC 14028 in Nutrient Agar

Sets	CGE in NA media	Amount of Inoculation	*Salmonella* Typhi
Set-I	5.0%	One loopful	Growth not inhibited
Set- II	10.0%	One loopful	Growth not inhibited
Set-III	15.0%	One loopful	Growth not inhibited
Set-IV	20.0%	One loopful	Growth not inhibited
Set-V	30.0%	One loopful	Growth not inhibited
Set- VI	40.0%	One loopful	Growth not inhibited
Set-VII	50.0%	One loopful	Medium growth
Set-VIII	60.0%	One loopful	Medium growth
Set-IX	70.0%	One loopful	Medium growth
Set-X	80.0%	One loopful	Medium growth
Set- XI	90.0%	One loopful	Medium growth
Set-XII	100.0%	One loopful	Growth completely inhibited
Set-XIII (Control)	Without CGE	One loopful	Huge Growth

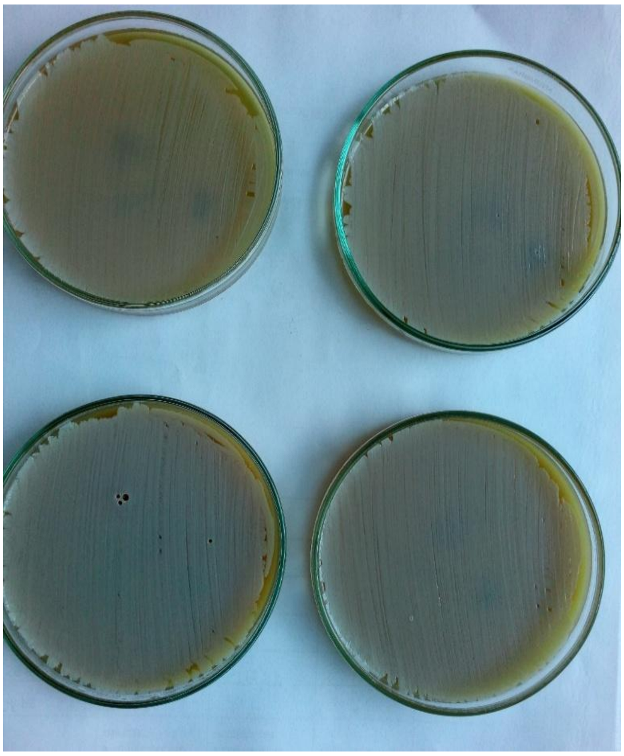

Figure I: Huge growth of *Salmonella* Typhi *in* CGE incorporated NA media

The antibacterial activity of ethanolic ginger extract (EGE) was evaluated against *Salmonella* Typhi using the disc diffusion method. Clear zones of inhibition were observed at all tested concentrations, indicating a dose-dependent antibacterial effect. At 25 µg/10 µl, the zone of inhibition measured 14 mm. Increasing the concentration to 50 µg/10 µl and 100 µg/10 µl resulted in larger inhibition zones of 30 mm and 33 mm, respectively, demonstrating enhanced antibacterial activity with higher extract concentrations. These results confirm that EGE exhibits significant inhibitory effects against *Salmonella* Typhi (Table 2).

Table 2: Zone of Inhibition of Ethanolic Ginger Extract Against *Salmonella* Typhi

Concentration of EGE per disc (10 µl)	Zone of Inhibition (mm)
25 µg	14
50 µg	30
100 µg	33

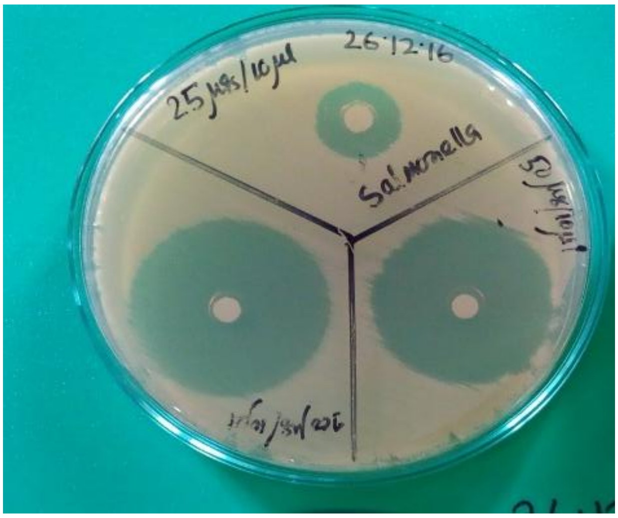

Figure II: Disc Diffusion showing *Salmonella typhi* is sensitive to EGE

The minimum inhibitory concentration (MIC) of ethanolic ginger extract (EGE) against *Salmonella* Typhi was determined using the broth dilution method. Visible bacterial growth was observed in test sets with EGE concentrations of 100–400 µg/ml, while no growth occurred in sets with concentrations of 500 µg/ml and above. This indicates that the MIC of EGE for *Salmonella* Typhi is 500 µg/ml (Set-V). Control experiments confirmed the validity of the results: Control-1, containing the stock EGE solution with bacterial inoculum, showed no growth; Control-2, containing nutrient broth with bacterial inoculum, showed abundant bacterial growth; and Control-3, containing nutrient broth without bacterial inoculum, showed no growth. These observations validate the inhibitory effect of EGE against *Salmonella* Typhi (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of EGE Against *Salmonella Typhi*

Sets	Concentration of EGE (µg/ml)	Growth of *Salmonella* Typhi
Set-I	900	No growth
Set-II	800	No growth
Set-III	700	No growth
Set-IV	600	No growth
Set-V	500	No growth
Set-VI	400	Growth
Set-VII	300	Growth
Set-VIII	200	Growth
Set-IX	100	Growth
Control-1	1000 (Stock EGE+Bacteria)	No growth
Control-2	Nutrient broth +Bacteria	Huge growth
Control-3	Nutrient broth only	No growth

The visible growth of *Salmonella* Typhi was observed in Set-I to Set-IV, while no growth occurred in Set-V and Set-VI. Therefore, the minimum inhibitory concentration (MIC) of Amikacin against *Salmonella* Typhi was determined to be 1.5 µg/ml (Set-V). Controls confirmed the validity of the experiment: Control-1 (nutrient broth without bacterial inoculum) showed no growth, while Control-2 (nutrient broth with bacterial inoculum) showed visible growth of the bacteria (Table 4).

Table 4: MIC of Amikacin Against *Salmonella* Typhi

No. of Sets	Concentration of Amikacin (µg/ml)	Growth of *Salmonella* Typhi
Set-I	0.25	Growth
Set-II	0.5	Growth
Set-III	0.75	Growth
Set-IV	1	Growth
Set-V	1.5	No growth
Set-VI	2	No growth
Control-1	NB medium only (no bacteria)	No growth
Control-2	NB medium + bacterial inoculum	Growth

The minimum inhibitory concentration (MIC) of Ethanolic Ginger Extract (EGE) against *Salmonella* Typhi was 500 µg/ml, whereas the MIC of Amikacin was 1.5 µg/ml. This observation indicates that Amikacin exhibits significantly stronger antibacterial activity than EGE against *Salmonella* Typhi (Table 5).

Table 5: Comparison of MIC of EGE and Amikacin Against *Salmonella* Typhi

Agent	MIC (µg/ml) for *Salmonella Typhi*
EGE	500
Amikacin	1.5

To confirm the inhibitory effects observed in previous experiments, subculture studies were performed using the effective concentrations of Crude Ginger Extract (CGE), Ethanolic Ginger Extract (EGE), and Amikacin. For *Salmonella* Typhi, the subculture results were consistent with the earlier observations: complete inhibition of growth was observed at 100% CGE, and the minimum inhibitory concentrations (MICs) of 500 µg/ml for EGE and 1.5 µg/ml for Amikacin showed no visible growth on nutrient agar plates.

Table 6: Subculture Study of Effective CGE, EGE, and Amikacin Against *Salmonella Typhi*

Test Organism	*Salmonella* Typhi
CGE (ml/100 ml)	100
No Growth in Subculture	Yes
EGE (µg/ml)	500
No Growth in Subculture	Yes
Amikacin (µg/ml)	1.5
No Growth in Subculture	Yes

Discussion

In the present study, the antibacterial potential of crude ginger extract (CGE), ethanolic ginger extract (EGE), and the standard antibiotic Amikacin was evaluated specifically against *Salmonella* Typhi ATCC 14028. The study provides scientific support for the traditional use of ginger as an antimicrobial agent and contributes to the growing body of evidence on plant-based alternatives against enteric pathogens.

CGE showed no inhibitory effect on *Salmonella* Typhi at concentrations from 5.0% to 40.0%, but growth inhibition began from 50.0% CGE and complete inhibition was observed only at 100.0% concentration. This indicates that *Salmonella* Typhi is relatively resistant to lower concentrations of crude extract, possibly due to the presence of its protective outer membrane, which reduces the permeability of bioactive compounds. Similar observations have been reported by previous researchers, who found that crude ginger extract generally requires higher concentrations to inhibit Gram-negative bacteria compared to Gram-positive organisms[8]. However, some variation exists in the literature, likely due to differences in ginger variety, extraction procedure, solvent used, and geographic factors influencing phytochemical composition.

The disc diffusion assay demonstrated that EGE exerted strong antibacterial activity against *S.* Typhi, producing clear inhibition zones in a dose-dependent manner. The zone of inhibition increased significantly from 14 mm at 25 µg/10 µl to 30 mm and 33 mm at 50 and 100 µg/10 µl, respectively. These findings suggest that ethanol extraction effectively concentrates the bioactive compounds of ginger, such as gingerols, shogaols, and other phenolic compounds, which are known to disrupt bacterial cell membranes and interfere with essential cellular processes. Compared with a previous study[9], which reported inhibition zones of 12 mm against *Salmonella* Typhi at 75.0% concentration, the current study demonstrated relatively larger zones of inhibition even at lower concentrations, indicating stronger antibacterial activity of the extract used in this investigation.

The minimum inhibitory concentration (MIC) of EGE against *Salmonella* Typhi was determined to be 500 µg/ml using the broth dilution method. This finding indicates that ethanolic ginger extract is bacteriostatic at higher concentrations and requires a relatively high dose to completely inhibit visible bacterial growth. Compared to a previous study[10] that reported a lower MIC value of 185 µg/ml for *Salmonella* Typhi, the higher MIC observed in the present study may be attributed to differences in bacterial strain, solvent extraction technique, experimental conditions, or the phytochemical composition of ginger due to environmental and geographical variation.

In contrast, the MIC of Amikacin against *Salmonella* Typhi was found to be 1.5 µg/ml, indicating that the antibiotic is significantly more potent than the ginger extract. This result is consistent with the well-known effectiveness of aminoglycoside antibiotics against Gram-negative bacteria like *Salmonella* Typhi. The large difference in MIC values highlights that while ginger possesses antibacterial properties, it cannot replace standard antibiotics in clinical treatment at present, but it may have potential as an adjunct therapy or supportive antimicrobial agent[10].

The subculture studies further confirmed that both EGE at 500 µg/ml and Amikacin at 1.5 µg/ml exhibited bactericidal effects against *Salmonella* Typhi, as no growth was observed upon transfer to fresh nutrient agar. Similarly, complete inhibition observed with 100.0% CGE also showed bactericidal nature. These findings are important because bactericidal activity is more desirable in the management of systemic infections like typhoid fever, where complete elimination of the pathogen is required[11,12]. Overall, the present study confirms that ginger extracts possess dose-dependent antibacterial activity against *Salmonella* Typhi, with ethanolic extract showing significantly higher efficacy compared to crude extract.

Conclusion

The study demonstrated that both crude and ethanolic ginger extracts exhibit antibacterial activity against *Salmonella* Typhi, with ethanolic extract showing stronger, dose-dependent inhibition. Amikacin was significantly more potent than the extracts. Subculture studies confirmed the bactericidal effects of all agents. These findings suggest that ginger extracts have potential as natural antimicrobial agents, though further research is needed to enhance their efficacy.

Acknowledgements
None

Conflict of Interest
None

Financial Disclosure
None

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Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

How to cite this article: Rahman MA, Nipu SA, Nahar DN, Dip RI, Mamun SH, Mostofa FB, Bashir MS, Rahman MA. In Vitro Evaluation of the Antibacterial Activity of Zingiber officinale (Ginger) Against Salmonella Typhi. Bangladesh J Infect Dis 2025;12(2):260-266

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Article Info
Received on: 1 September 2025
Accepted on: 20 October 2025
Published on: 1 December 2025

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