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UNVEILING THE ANTIBACTERIAL ACTIVITY OF KAEMPFEROL AGAINST *SALMONELLA TYPHI*: A COMBINED IN VITRO AND IN SILICO APPROACH

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Abstract

Typhoid fever, the disease caused by Salmonella typhi serovar Typhi (S. typhi), remains a major question of the global population and especially in the developing world. The development of multidrug-resistant strains has led to the decline in the effectiveness of the conventional antibiotics, and the development of alternative antimicrobial agents is in demand. Kaempferol is a commonly occurring flavonoid that has been reported to have a wide range of pharmacological properties such as antimicrobial activity. The current research was conducted to assess the antibacterial properties of kaempferol against S. typhi through a combined in silico and in vitro research design. Minimum inhibitory concentration (MIC) and time-kill kinetic inhibition were used to determine in vitro antibacterial activity. Molecular docking of kaempferol with key proteins of the bacterial targets were done in silico. Kaempferol was also a moderate antibacterial agent with an MIC value of 64 µg/mL and it showed concentration-dependent growth inhibition. Molecular docking was found to have positive binding affinities of kaempferol to DNA gyrase, which are fundamental to the survival of bacteria. These results indicate that kaempferol has antibacterial properties against S. typhi due to its interference with essential processes of the bacterium and can be considered a good lead in the design of novel antibacterial agents.

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INTRODUCTION

Typhoid fever is among the leading infectious diseases in most areas in the world particularly in places with poor sanitation and availability of clean water (Thalia, 2024) *Salmonella enterica* serovar Typhi (*S. typhi*) is a Gram-negative, facultative intracellular pathogen, the cause of which carries out the disease and can cause severe systemic infection (Turicchi & Bongiovanni, 2025). It is estimated by the global health that typhoid fever has millions of cases annually resulting into massive morbidity and mortality. It has also become more difficult with the emergence of multidrug-resistant (MDR) and highly drug-resistant (XDR) strains of *S. typhi* in the management of the typhoid fever therapeutically (Zakir et al., 2021).

The resistance to the fluoroquinolones has been widely documented to be reduced by the first-line antibiotics such as chloramphenicol, ampicillin and trimethoprimsulfamethoxazole (Brar et al., 2020). These problems suggest the necessity to work out alternative antimicrobial methods in the nearest future. The use of natural products in the discovery of drugs has a long tradition and more so in the establishment of antimicrobial agents (Abdel-Razek et al., 2020). Flavonoids are a broad family of polyphenolic compounds, which are synthesized by fruits, vegetables and medicinal plants and remain of considerable interest to the biology of their numerous activities, which include antioxidant, anti-inflammatory, anticancer, and anti-microbial (Al-Khayri et al., 2022).

Kaempferol (3, 4, 5, 7-tetrahydroxyflavone) is a flavonoid that is found in high amounts in foods that include onions, kales, tea, and berries (Rakha et al., 2022). A number of studies have been able to reveal its antibacterial effect on Gram-positive and Gram-negative bacteria, but very little information can be located on its action on *S. typhi* and its mechanism of action. As such, the purpose of the study was to examine the antibacterial efficacy of kaempferol against *S. typhi* using an in vitro/in silico methodology to deliver mechanistic information and justify its use as a new antibacterial lead drug.

Materials and Methods

Chemicals and Reagents

Kaempferol was purchased commercially at 98 percent purity or more. Antibacterial assays were done in Muller Hinton broth (MHB) and Muller Hinton agar (MHA). A reference antibiotic was Ciprofloxacin. The reagents were of the analytical grade.

Bacterial Strain

This study was done using a reference strain of *Salmonella typhi*. The strain was kept on MHA slants and sub-cultured before subjecting them to experimentation

In Vitro Antibacterial Activity

Determination of Minimum Inhibitory Concentration (MIC)

The broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) was used to determine the MIC of kaempferol against *S. typhi*. Kaempferol was diluted in serial (1 512) 2-folds in MHB. A standard bacterial inoculum (about 1×10^6 CFU/mL) was introduced in each well and incubated at 37 ^{0}C after 18-24 hours. The MIC was determined as the lowest level of kaempferol to fully suppress the bacterial growth as seen (Chikezie, 2017).

In Silico Studies

Ligand and Protein Preparation

Kaempferol was a three-dimensional structure that was retrieved in chemical databases and minimized in energy. The Protein Data Bank was used to obtain target proteins that included DNA gyrase subunit A, DNA gyrase subunit B of S. typhi. The molecules of water and other non-essential ligands were eliminated, and proteins were made ready to docking (Parveen et al., 2025).

Molecular Docking

AutoDock Vina was used to carry out Molecular docking in order to determine the binding affinity and binding patterns between kaempferol and the bacterial targets of interest. Boxes were

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established that covered active site of the proteins. The values of docking were presented as binding energy (kcal/mol) and interaction profiles of the same were examined (Owoloye et al., 2022).

Results

In Vitro Antibacterial Activity

S. typhi was inhibited by kaempferol and its MIC was 64 µg/mL. Ciprofloxacin showed much lower values of MIC which acts as a confirmation of the assay.

Molecular Docking Results

Kaempferol showed good binding affinities to DNA gyrase B (-8.1 kcal/mol). Hydrogen bonding, hydrophobic and π-π stacking interactions were also identified with the important active-site residues suggesting the possibility of inhibiting these critical proteins (Figure 1 and 2).

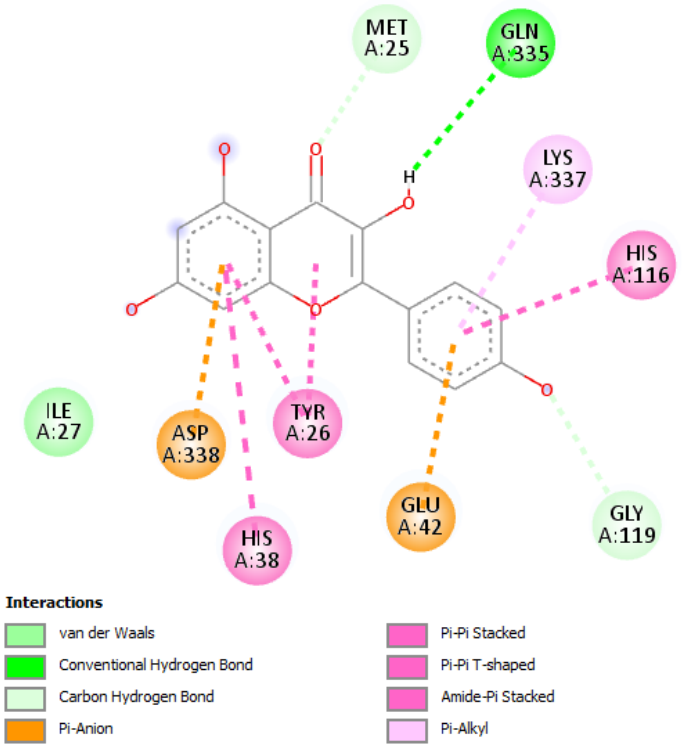


Figure 1: 2 D structure of *Salmonella typhi* protein bind with kaempferol

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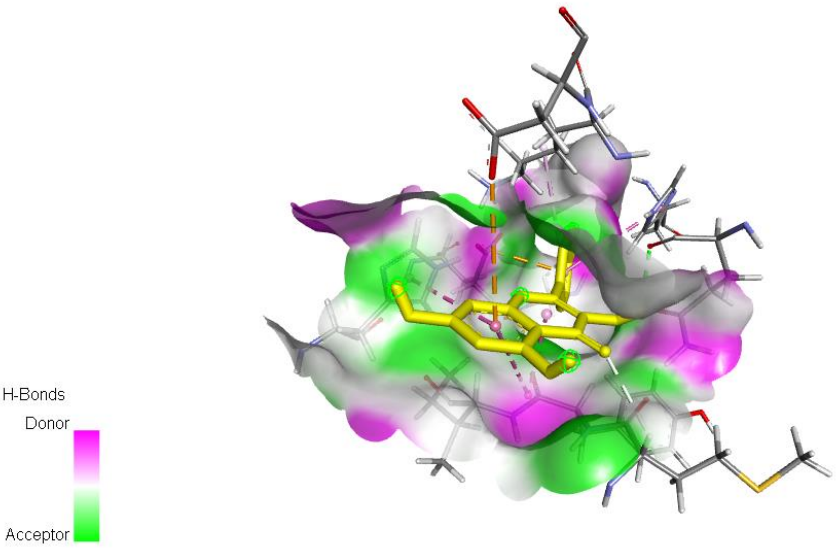


Figure 2: 2 D structure of *Salmonella typhi* protein bind with kaempferol

Discussion

The current research established that kaempferol has moderate antibacterial effects over S. typhi due to MIC and time kill kinetic studies. Its activity was not as high as that of ciprofloxacin, but the natural origin and multitarget potential of kaempferol are its positive factors, which makes it a good candidate to develop (Kakoullis et al., 2021). The in silico results were highly consistent with the in vitro ones since they showed the presence of stable interactions between kaempferol and

DNA gyrase which is a key enzyme in the DNA replication of bacterium (Harini et al., 2024). DNA gyrase inhibition also interferes with the normal functioning of the cell and eventually the growth of the cell is inhibited (Rajendram et al., 2014). Also, the contacts with the outer membrane proteins indicate that kaempferol can influence the permeability of membranes and the delivery of nutrients (Lou et al., 2025).

The flavonoid compounds like kaempferol are known to have antibacterial effects in several different ways; enzyme inhibition, membrane destabilization, metallic ion chelation, and through the induction of oxidative stress (Simunkova et al., 2021). Such multitarget mode of action will lower the chances of development of resistance, which is a significant benefit compared to traditional antibiotics (Bangar et al., 2023). The research still has limitations, such as using a single bacterial strain, lack of cytotoxicity, and in vivo research despite the positive results. Future studies must involve the efficacy of kaempferol against clinical isolates of MDR, the synergistic effect of the compound with antibiotics, and pharmacokinetics and toxicity studies.

Conclusion

This paper has provided a comprehensive in vitro and in silico evidence on the antibacterial activity of kaempferol on *Salmonella typhi*. Although the potency is moderate, the regularity of the interaction between kaempferol and necessary bacterial targets is an indication that the compound should be used as a lead compound to develop novel antibacterial agents. Further optimization and experimental validation is warranted to bring out the greatest therapeutic potential of it.

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