Palestinian Medical and Pharmaceutical Journal



Development and Computational Analysis of Polyherbal Chewable Lozenges for Effective Management of Mouth and Throat Infections

Zaid Khan^{1,*}, Ramya C V¹, Deepthi Swapna PR² & Vishwanath B A² Received: 15th Dec. 2024, Accepted: 9th Apr. 2025, Published: ××××, 202X, DOI: ××××

Accepted Manuscript, In Press

Abstract: This study aimed to develop and evaluate polyherbal chewable lozenges for the treatment of mouth and throat infections, integrating traditional herbal knowledge with modern pharmaceutical and computational techniques. The lozenges were formulated using Karpuravalli (Coleus amboinicus), Ginger (Zingiber officinale), Cardamom (Elettaria cardamomum), and Clove Oil (Syzygium aromaticum), and their quality, efficacy, and therapeutic potential were rigorously assessed. Quality control tests, including weight variation, friability, moisture content, and dissolution, confirmed the lozenges' compliance with pharmaceutical standards. The dissolution profile demonstrated a cumulative drug release of 91.26% within 25 minutes, indicating efficient bioavailability. Antimicrobial activity tests revealed significant efficacy against Staphylococcus aureus (15 mm inhibition zone) and Escherichia coli (25 mm inhibition zone), with E. coli showing greater susceptibility. Molecular docking studies identified Rosmarinic Acid and Beta-Sitosterol as key bioactive compounds, exhibiting strong binding affinities with bacterial enzymes such as Dihydrofolate Reductase (DHFR) and UDP-N-Acetylglucosamine Enolpyruvyl Transferase, suggesting potential inhibitory mechanisms. Pharmacokinetic and drug-likeness evaluations using Lipinski's Rule of Five and Swiss ADME highlighted favorable properties for compounds like Carvacrol and Gingerol, while bioactivity scores indicated diverse interaction potentials. The study underscores the lozenges' adherence to quality standards and their potential as effective antimicrobial agents. However, further optimization of formulation strategies, mechanistic studies, and clinical trials are necessary to validate their therapeutic efficacy and safety. This research contributes to the growing body of evidence supporting the use of polyherbal formulations in modern therapeutics, offering a natural and scientifically validated alternative for managing oral and throat infections.

Keywords: Polyherbal lozenges, antimicrobial activity, quality control, docking analysis, phytochemicals, ADME, bioactivity.

Introduction

Definition

Lozenges are a form of medicinal or dietary supplement designed for oral administration, gradually dissolving within the mouth. They are commonly employed to alleviate discomfort associated with sore throats, coughs, and various respiratory ailments. [1] Lozenges can manifest in diverse formats, encompassing medicinal pastilles as well as therapeutic and preventive dentifrice. [2]

Throat Infections and Thrush

Throat infections can arise from various microorganisms, encompassing bacteria and viruses. Streptococcus is the predominant bacterial agent responsible for throat infections, whereas the principal viral culprit is the influenza virus. [3] Apart from pathogenic microorganisms, throat infections can also be induced by non-infectious elements such as allergies, physical injury, and malignant growth. [4] Factors that increase the susceptibility to throat infections encompass smoking, susceptibility to air pollutants, and compromised immune systems. [5]

Significance of Herbal Lozenges as a Potential Treatment Option

Herbal lozenges represent a viable therapeutic avenue for an array of afflictions and conditions, encompassing ailments such as sore throats, coughs, and oral ulcers. These are compacted formulations comprising one or multiple remedies, typically within a tastefully seasoned and saccharine substrate, with the aim of gradual dissolution or disintegration within the oral cavity. [6]

Benefits of herbal lozenges

Herbal lozenges extend the duration that medication remains in the mouth, enhancing absorption, reducing stomach irritation, and avoiding initial metabolic processing. They provide an appealing drug delivery method and hold a significant position in the pharmaceutical industry due to their numerous benefits. Herbal lozenges are versatile, suitable for both localized and systemic treatments, and can accommodate a wide range of active ingredients [7].

These lozenges serve as a compelling alternative to conventional medications, as they can be developed using organic additives. These natural components offer advantages such as being non-toxic, cost-effective, and biodegradable, making them a preferable choice over synthetic counterparts [8].

Herbal treatments, including lozenges, play a crucial role in managing diabetes, a common and serious metabolic condition. According to the World Health Organization (WHO), 80% of people in developing countries rely on traditional medicines due to their affordability, effectiveness, and fewer side effects compared to modern drugs [9]. Herbal lozenges, with their

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¹ Department of Pharmacy Practice, Faculty of Pharmacy, Aditya Bangalore Institute of Pharmacy Education and Research, Banglore, India *Corresponding author email: zaidkhan9515@gmail.com

² Department of Pharmaceutics, Faculty of Pharmacy, Aditya Bangalore Institute of Pharmacy Education and Research, Banglore, India

natural composition and therapeutic potential, align well with this preference for traditional and holistic healthcare solutions.

Benefits of lozenges as a drug delivery system

Lozenges are a novel oral medication delivery system that provide unique therapeutic benefits due to their slow dissolution and prolonged interaction with oral tissues. Their primary advantage lies in enabling localized drug delivery to specific areas of the mouth and throat, ensuring sustained therapeutic concentrations while reducing systemic exposure [10]. Unlike conventional oral solutions or sprays, lozenges maintain intimate contact with the oral mucosa for typically 5-10 minutes, creating a drug reservoir that allows for more consistent and prolonged release [11]. This delivery method partially bypasses first-pass metabolism through mucosal absorption, potentially enhancing the bioavailability of certain drugs, reducing the required dosage, and minimizing systemic side effects.

Lozenges also offer better palatability, ease of administration without water, and distinct advantages for pediatric, elderly, and dysphagic patients who face challenges with traditional dosage forms. Recent technological advancements have further expanded the applications of lozenges, establishing them as a sophisticated and patient-friendly option for treating conditions of the upper respiratory tract and oral cavity [12]. These advancements include innovative formulations that provide modified release profiles, improved stability, and enhanced manufacturing efficiency [13].

General introduction on microbes induced mouth and throat infection with signs, symptoms and etiology

People of all ages are susceptible to microbial infections of the mouth and throat, which pose a serious threat to global health. Numerous pathogens, such as bacteria, fungi, and viruses, can cause these infections. Although the majority of infections are viral, such as those caused by influenza, adenovirus, and rhinovirus, bacterial infections especially those caused by Streptococcus species, which cause 5-15% of adult cases and up to 30% of pediatric cases also play a significant role in the burden of disease [14]. Oral thrush, which is mainly caused by Candida albicans, is another risky fungal infection, particularly for those with weakened immune systems.

Narrow-spectrum antibiotics are essential to treat bacterial infections, notably those brought on by Group A Streptococcus (GABHS), in order to reduce symptoms, minimize complications, and lower the risk of transmission. The necessity of early identification and management is underscored by the fact that GABHS infections can result in significant illnesses including post-streptococcal glomerulonephritis, rheumatic heart disease, and peritonsillar abscess if therapy is not administered [15,16]. On the other hand, supportive treatment, such as analgesics, hydration, and warm saline gargles, is the most effective approach to treat viral infections, which do not require antibiotics.

Recent studies have brought attention to the oropharyngeal microbiome's role in infection susceptibility, indicating that preserving a healthy microbial environment is essential for infection prevention. This strategy supports efforts to develop vaccines against important diseases, antimicrobial stewardship, and standard hygiene practices [17, 18].

One of the most common bacterial pathogens that causes throat infections is Staphylococcus aureus. According to studies, the throat microbiota commonly contains S. aureus, including its methicillin-resistant strain (MRSA), with a prevalence of 3.1% for MRSA and 23.1% for S. aureus [19]. These bacteria's colonization can raise the risk of illness, especially in people with weakened immune systems. Furthermore, whereas Escherichia coli is mostly linked to respiratory, urinary, and gastrointestinal

tract infections, it has also been linked to few instances of mouth infections, including mandibular osteomyelitis [20].

An integrated approach that incorporates early detection, targeted treatment, microbial balance preservation, and preventive strategies can greatly reduce the burden of microbial infections in the mouth and throat, ultimately improving overall health outcomes, thanks to advancements in research and diagnostic tools [21,22].

Herbal ingredients effectiveness on throat and mouth infections

The use of herbal remedies for throat and mouth infections has been a traditional practice in many cultures. Several studies have highlighted the potential effectiveness of herbal products in treating oral and throat ailments. For instance, herbal gargles have been reported to have antimicrobial, anti-inflammatory, and anti-plaque properties, with ingredients such as Tulsi, Turmeric, Clove, Fennel, Betel leaves, Pudina, Ginger, and Liquorice showing effective activity against oral pathogens.[23] Detailed overview of Mechanism of absorption of chewable lozenges into the tissues is shown in Fig.1

Mechanism of Slow Dissolution









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Figure (1): Mechanism of absorption of lozenges through active diffusion and destruction of bacteria by active ingredients

Selection criteria for herbal ingredients used in the formulation of lozenges

Coleus amboinicus (Karpooravalli) Powder

Coleus amboinicus, a medicinal plant traditionally used for treating various diseases, has shown antimicrobial properties, The plant has been found to contain phenolic compounds such carvacrol, flavonoids, rosmarinic, caffeic acid, and as chlorogenic acid.[24] Thymol, a naturally occurring compound present in Coleus amboinicus, has been identified for its inherent antimicrobial attributes. An investigation focused on multidrugresistant strains of E.coli and S.aureus demonstrated that the methanol extract derived from the leaves of Coleus amboinicus manifested noteworthy antibacterial efficacy against these pathogens. The minimum inhibitory concentrations (MIC) recorded for E.coli and S. aureus were 15.6 mg and 7.8 mg, respectively.[25]

Ginger powder (Zingiber officinale)

A separate investigation documented that a nanoemulsion, composed of a blend of extracts from garlic and ginger, displayed antimicrobial efficacy against E. coli and S. typhi.[26] The active

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compound in ginger powder that exhibits antimicrobial properties are Gingerol and gingerol related compound, Paradol, Zingerone, Zerumbone.[27]

Cardamom powder (Elettaria cardamomum)

Cardamom, particularly its essential oil and seed powder extract, has been identified as harboring antimicrobial characteristics. Investigations have elucidated the antibacterial potential of cardamom against a spectrum of microorganisms, encompassing S. aureus, S. typhi, Streptococci, and Streptococcus Mutans.[28] Cardamom extracts contain bioactive compounds such as flavonoids and sterols, exemplified by βsitosterol, which have been identified as contributors to the antimicrobial attributes. Additionally, the essential oil of cardamom comprises terpenoids, including a-pinene, β-pinene, limonene, and linalool, recognized as active constituents.[29] These compounds are implicated in various health-promoting properties of cardamom, such as antibacterial, antioxidant, antidiabetic, anti-inflammatory, and anti-tumor effects.[30] Furthermore, the presence of methylencholanthrene in cardamom has been associated with its antimicrobial potential.[31,32]

Synergistic Potential of Coleus amboinicus, Ginger powder and Cardamom powder

Antimicrobial Effects: The antimicrobial qualities of cardamom and ginger are well-known, and they may be strengthened by combining them with karpooravalli, a plant that has been shown to be effective against a variety of germs, including Staphylococcus aureus and Escherichia coli. These herbs' combined effects may offer a comprehensive defense against illnesses.

Anti-inflammatory Activity: The anti-inflammatory qualities of cardamom and ginger can be enhanced by the anti-inflammatory effects of karpooravalli. Due to their synergistic effects, studies on comparable herbal combinations, such as karpooravalli with mint and cinnamon, have demonstrated strong anti-inflammatory effectiveness.[33]

Antioxidant Properties: The Zingiberaceae family, which includes ginger and cardamom, is rich in antioxidants that can protect against oxidative stress. Karpooravalli also exhibits antioxidant activity, potentially enhancing the overall protective effects of the combination.[34]

Traditional Uses Supporting the Combination

Digestive Health: Ginger and cardamom have long been used to treat digestive problems. Their medicinal efficacy in treating respiratory and gastrointestinal issues may be increased when combined with karpooravalli.[35]

Antimicrobial Action: These herbs' selection for antimicrobial applications is supported by their historical usage in the treatment of infections and the encouragement of wound healing. Research on comparable herbal combinations has demonstrated encouraging outcomes in terms of antibacterial effectiveness and wound healing. [36]

Clove oil (Syzygium aromaticum)

Clove oil has been recognized for its antimicrobial effectiveness against a wide range of microorganisms, including bacteria, fungi, and viruses. Traditionally used as an antiseptic for oral infections and as a preservative and antimicrobial agent in food, clove oil's potent antimicrobial properties are primarily due to its active ingredient, eugenol, which exhibits strong antimicrobial activity. Research suggests that clove oil shows promise as a potential antimicrobial agent for external applications [37].

Mishri (Stone sugar)

Mishri, also known as rock sugar, is a crystallized form of sugar widely used in Indian culinary practices and traditional medicine. Some references suggest that natural sweeteners like mishri may offer potential health benefits compared to refined sugar, citing a lower glycemic index and higher mineral content. It is often incorporated into Ayurveda due to its purported medicinal properties. However, it is important to emphasize that further research is necessary to substantiate these claims [38, 39]. All the herbal ingredients are summarized in Fig. 2.

Molecular docking

Molecular docking is a computational method employed to elucidate the interactions between a small molecule (ligand) and a protein (target) at an atomic resolution.[40] This technique is pivotal in structural molecular biology and computer-aided drug design, as it provides insights into the binding mechanisms of ligands to proteins, which is fundamental for drug discovery and optimization.[41] The principal aim of molecular docking is to predict the optimal orientation of a ligand when it binds to a target protein, which is crucial for assessing the binding affinity and stability of the resulting complex. Such insights are particularly valuable in drug development, as they facilitate the design of effective therapeutic agents.[42]



Figure (2): Ingredients used in the development of lozenges, highlighting the key components that contribute to their formulation and therapeutic properties.

Computational analysis is integral to the assessment of polyherbal chewable lozenges, offering a systematic approach to understanding the interactions and effectiveness of the herbal components. This analysis can be segmented into several crucial areas

Molecular Interaction Studies

Computational techniques, such as molecular docking and dynamics simulations, allow researchers to predict interactions between active compounds in lozenges and biological targets. For instance, these methods have been used to identify potential inhibitors of specific enzymes, like the SARS-CoV-2 main protease, by analyzing the binding affinities of herbal compounds. This approach helps in selecting the most effective ingredients for lozenge formulation [43].

Drug-Likeness and ADME Profiling

Assessing the drug-likeness of herbal compounds involves evaluating their Absorption, Distribution, Metabolism, and Excretion (ADME) properties. Computational tools can predict these properties, ensuring that the selected herbal ingredients are not only effective but also safe and bioavailable when incorporated into lozenges. Drug-likeness analyses confirm that herbal compounds adhere to established pharmacological criteria, validating their suitability for therapeutic use.[44]

Quality Control and Safety Assessment

Computational analysis supports the quality control of herbal formulations through pattern recognition and chemometric techniques. These methods assess the chemical profiles of herbal ingredients to ensure consistency and authenticity, which is crucial for maintaining lozenge efficacy and safety. Additionally, toxicological assessments using computational tools can identify potential adverse effects of the herbal compounds, ensuring the final product is safe for consumption.[45]

Formulation Optimization

Computational modeling facilitates the optimization of polyherbal lozenge formulations by simulating various ingredient combinations and concentrations. This process enhances therapeutic efficacy while minimizing undesirable interactions among herbal components. Computational analysis serves as a powerful tool in the development and evaluation of polyherbal chewable lozenges, improving their efficacy, safety, and overall quality through comprehensive molecular insights and predictive modeling [46, 47].

Computational methods specifically contribute to the development and evaluation of herbal formulations

Molecular docking predicts how bioactive compounds from herbs bind to specific biological targets, such as enzymes or receptors. This makes it easier to understand the molecular mechanisms behind their therapeutic advantages. For example, docking studies have been utilized to ascertain the interactions between phytochemicals and target proteins linked to disorders such as Alzheimer's or inflammation.[48] Understanding the synergistic interactions between different components is also helpful in order to optimize the efficacy of herbal formulations.[49]

ADME Description:

ADME profiling evaluates the pharmacokinetics of herbal substances, including their distribution, metabolism, excretion, and absorption properties. This ensures that when used therapeutically, the bioactive substances are both safe and effective.[50] Early on in the drug development process, bioavailability and toxicity may be predicted using computational methods.[51]

Mechanistic Understanding:

The stability and behavior of chemicals generated from herbs under physiological settings may be thoroughly understood by combining molecular docking with other computational techniques, such as molecular dynamics simulations.[52]

As seen by research on traditional Chinese medicine formulations such as Tian-Ma-Gou-Teng-Yin for Alzheimer's disease, systems pharmacology techniques combine networkbased predictions with bioinformatics to investigate intricate relationships between herbal components and several biological targets.[53]

Artificial Intelligence (AI) Integration:

Large datasets are analyzed by AI-driven methods like machine learning and neural networks to find active ingredients, forecast treatment outcomes, and improve formulations. These strategies speed drug discovery by lowering time and cost compared to classic experimental procedures. [54,55]

Research Gap

Current research primarily focuses on the formulation, development, and assessment of polyherbal lozenges, yet computational methods such as pharmacokinetics modeling and molecular docking are not widely utilized to predict the synergistic effects of herbal components or optimize formulation design [56, 58]. Most studies emphasize physicochemical and in vitro evaluations, but there is a lack of comprehensive clinical trials to validate the efficacy and safety of polyherbal lozenges in real-world settings for treating mouth and throat infections [59]. Additionally, research on chewable lozenges, which could enhance patient compliance, particularly among pediatric and geriatric populations, is significantly scarcer compared to studies on hard lozenges [58]. While polyherbal formulations are recognized for their synergistic effects, there is limited research on measuring or enhancing these interactions to improve therapeutic outcomes [57]. Furthermore, few studies compare the efficacy of polyherbal lozenges with conventional synthetic treatments, such as antibacterial or analgesic lozenges, leaving a gap in understanding their relative advantages [58].

Comparison of Herbal Lozenges with Conventional Treatments

Herbal lozenges and conventional treatments each offer distinct advantages and limitations for managing symptoms like sore throat and cough. Herbal lozenges, often containing natural ingredients such as honey, ginger, or echinacea, provide soothing and anti-inflammatory effects with fewer side effects, making them a preferred choice for individuals seeking holistic or natural remedies. For example, honey-based lozenges have been shown to reduce cough frequency and improve sleep quality in children [59]. However, their efficacy can vary due to inconsistent concentrations of active ingredients, and they may trigger allergic reactions in sensitive individuals.

In contrast, conventional treatments, which typically contain synthetic compounds like benzocaine or dextromethorphan. offer rapid and predictable relief by numbing the throat or suppressing cough reflexes. Studies indicate that dextromethorphan is particularly effective in reducing cough severity [60]. However, conventional treatments may pose risks such as drowsiness, gastrointestinal discomfort, or rare but serious side effects like methemoglobinemia with benzocaine overuse [61]. Accessibility and cost also differ, with herbal lozenges being more affordable and culturally accepted in certain regions, while conventional treatments are often covered by insurance in developed countries but may be cost-prohibitive Ultimately, the choice between herbal and for some [62]. conventional treatments depends on symptom severity, patient preference, and cultural context. Herbal options appeal to those prioritizing natural remedies, while conventional treatments are favored for their rapid and reliable action.

Methods

All the herbal ingredients were purchased from local supermarkets in Bangalore, with some ordered online. A summary of the ingredients is provided in Table 1 and Fig. 2.

Table (1): Ingredients used in the preparation of granules, detailing the quantities in milligrams, grams, and the total amount required for 100 tablets.

SI.no	Ingredients	Milligrams	Grams	For 100 tablets
1.	Karpooravalli Powder	100	0.1	10 g
2.	Ginger Powder	100	0.1	10 g
3.	Elachi Powder	50	0.05	5 g
4.	Starch	20	0.02	2 g
5.	Talc	5	0.05	5g
6.	Magnesium Sterate	5	0.05	5g
7.	Mishri [Sugar Candy]	220	0.22	22g
8.	Clove Oil	0.5 [ml]	0.5[ml]	0.5 [ml]

Preparation of granules

The wet granulation method was employed for the preparation of granules. Each ingredient karpooravalli (Coleus amboinicus), ginger powder (Zingiber officinale), and elachi powder (Elettaria cardamomum) was meticulously weighed individually and thoroughly mixed using a mortar and pestle. A 2% starch paste was carefully prepared and added incrementally to the mixture. To enhance the formulation, 0.5 ml of clove oil (Syzygium aromaticum) was incorporated. The combined mixture was diligently stirred to form a paste, which was then sieved through a 22-mesh screen. The resulting granules were dried to complete the process.

Tablet Punching

The tablet punching process for 500 mg granules in a tablet die-punching machine involves a meticulous series of steps to ensure precision and consistency. Initially, 500 mg of granules is accurately weighed to guarantee uniformity and consistent tablet weight. The tablet press is thoroughly cleaned and calibrated, and the appropriate die for 500 mg tablets is set up. The granules are loaded into the hopper, and compression parameters, including pressure and dwell time, are configured. The tablet press is then activated, compressing the granules into tablet form. The resulting tablets are collected and securely packaged. After each batch, the tablet die-punching machine is thoroughly cleaned to prevent cross-contamination. The entire process is validated to ensure compliance with regulatory standards and consistent tablet quality, adhering to academic and pharmaceutical industry norms. A summary of the methodology used in the development of chewable herbal lozenges is illustrated in Fig. 3.



tep 1: Rock





ep 2: All the herbal Step 1: Rock sugar/ Candy sugar/ Mishri, ingredients mixed with

Step 3 : cohesive was formed following ing method.





Step 6: Granules were punched in to chewable lozenges

Step 5 : Large granules were passed through the sieve to reduce its size

Figure (3): Methodology used in the formulation of polyherbal lozenges, illustrating the step-by-step process from powdering rock sugar to forming cohesive mass, drying, sieving, and punching into chewable lozenges.

Quality Control Test for Chewable Lozenges

Weight Variation Test

The weight variation test for chewable lozenges was conducted to assess the uniformity of weight among a sample of 20 tablets. This test aimed to ensure the consistency and accuracy of dosage in the pharmaceutical product. The procedure involved individually weighing each tablet, recording the results, and calculating the average weight. The acceptable weight variation limits were determined based on Indian pharmacopeial standards. Deviations beyond these specified limits could indicate issues with the manufacturing process, formulation, or ingredient uniformity. By performing this test, the quality, safety, and efficacy of the chewable lozenges were evaluated. The results of this analysis are discussed in the results section.

Friability Test

The friability test for chewable lozenges is a quality assessment procedure conducted to evaluate the resistance sample of 20 tablets to abrasion during handling and transportation. The test is executed using a friability apparatus, where the tablets are subjected to repeated tumbling inside a drum. Initially, 20 tablets are carefully weighed and placed in the drum. The drum is then rotated for 100 revolutions. Following the test, the tablets are re-weighed, and the percentage weight loss is calculated. The acceptable friability limits are defined by pharmacopeial standards or specific product requirements. A higher percentage weight loss may indicate a tendency for the chewable lozenges to break or crumble, affecting dosage accuracy. This test serves as a crucial parameter in ensuring the robustness and stability of chewable lozenge formulations.

Hardness Test

The hardness of the formulated polyherbal chewable lozenges was assessed using a Pfizer Hardness Tester to determine their mechanical strength. A single lozenge was placed between the tester's jaws, and force was gradually applied until the tablet fractured. The hardness (crushing strength) was recorded in kilograms per square centimeter (kg/cm²).

Each batch of lozenges was tested in triplicate (n=3), and the mean ± standard deviation (SD) was calculated. This test ensured that the lozenges possessed adequate mechanical strength to withstand handling and packaging while allowing gradual dissolution in the oral cavity.

Moisture Content / Water Content

The moisture content or water content was determined using the Karl Fischer apparatus. The Karl Fischer titration method was applied, employing the volumetric (direct titration) technique for accurate measurement.

Dissolution test

To establish a dissolution testing procedure for chewable lozenges to determine the percentage drug release under simulated physiological conditions. The experiment utilized USP Dissolution Apparatus II (Paddle) with a pH 5.8 artificial saliva solution as the dissolution medium. The temperature of the buffer solution was maintained at 37 ± 0.5°C within the dissolution apparatus. Calibration of the apparatus was performed according to the manufacturer's instructions to ensure proper paddle rotation speed.

For sample preparation, a representative number of chewable lozenges from the batch were selected, and their individual weights were recorded. The percentage drug release was determined using Microsoft Excel software. Dissolution vessels were prepared by placing one lozenge in each vessel and adding 900 mL of pH 5.8 artificial saliva buffer. The dissolution testing began by initiating the apparatus at a specified paddle rotation speed. Samples were collected at regular intervals (e.g., 5, 10, 15, 20, 25 minutes) using a pipette. To maintain a constant volume, the withdrawn samples were replaced with an equal volume of fresh dissolution medium. The collected samples were filtered through filter paper.

Culture sensitivity test

To evaluate the efficacy of antimicrobial agents, a culture sensitivity test was conducted. Sterile nutrient agar plates were prepared by autoclaving. Once ready, the plates were inoculated with specific microbial cultures using a sterile cotton swab or inoculating loop, ensuring even distribution of the inoculum across the agar surface. Prepared herbal lozenges containing known concentrations of the formulated herbal lozenges were then affixed onto the inoculated plates using sterile forceps. The

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plates were incubated under appropriate conditions (e.g., specific temperature and duration) to allow microbial growth. After incubation, the zones of inhibition around each lozenge were observed and measured using a ruler or caliper. The size of these zones indicated the effectiveness of the antimicrobial agents against the tested microorganisms.

Docking Analysis

Selection and preparation of ligand

Sixteen bioactive compounds from plant sources, including Indian borage (Coleus amboinicus, synonym Plectranthus amboinicus), ginger (Zingiber officinale), green cardamom (Elettaria cardamomum), and clove (Syzygium aromaticum), were collated from public databases and published research papers. The 3D structures of these compounds were downloaded in SDF format from (https://pubchem.ncbi.nlm.nih.gov/) A summary of the 3D structures of all 16 ligands used in this study is provided in Fig. 4.



Figure (4): Ligands opted for Docking (A)Carvacrol, (B)Rosmarinic acid, (c)Caffeic acid, (D)Thymol, (E)Gingerol, (F)Paradol, (G)Zingerone, (H)Zerumbone, (I)Shogaol, (J)Beta Sitosterol, (K)α-Terpinyl acetate, (L)Sabinene, (M)Linalool, (N)α-Pinene, (O)Eugenol, (P)β-Caryophyllene.

Protein preparation

The 3D structures of Staphylococcus aureus S1: DHFR (dihydrofolate reductase enzyme from Staphylococcus aureus type S1) and UDP-N-acetylglucosamine enolpyruvyl transferase were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) with PDB IDs 2W9T for Staphylococcus aureus S1:DHFR and 1UAE for UDP-N-acetylglucosamine enolpyruvyl transferase of E. coli. Using UCSF ChimeraX 1.8, all non-protein parts were removed, and polar hydrogen atoms and Kollman's charges were added. The prepared proteins were saved in PDB format.

Docking Analysis

The selected chemical compounds and protein structures were uploaded into the virtual screening software, PyRx. Energy minimization was performed using the conjugate gradient algorithm in conjunction with the Universal Force Field (UFF), with a total of 200 steps, and updates occurring at each step. The minimization process was programmed to terminate when the energy difference dropped below 0.1 kcal/mol.

Subsequently, both the chemical compounds and protein structures were converted to the. pdbqt format using the Open Babel tool integrated within PyRx. The active binding site grid

box was generated using the forward option in PyRx, with the grid box size and coordinates adjusted either by manipulating the boundary lines or by directly inputting specific values into the designated fields.

The conformational search algorithm employed in PyRx was the Lamarckian genetic algorithm, and the docking procedure used in this study followed a semi-flexible docking approach.

Rule of Five

Lipinski's Rule of Five (RO5) is a widely used criterion for evaluating the drug-likeness of a compound, which is a critical step in drug discovery. This rule helps to predict whether a compound is likely to exhibit oral bioavailability. In this study, ligands were assessed for compliance with RO5 using the Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp).Bioactive compounds demonstrating binding energies comparable to those of standard drugs were further analyzed using this criterion.

In silico ADME analysis

Pharmacokinetic parameters, including Absorption, Distribution, Metabolism, and Excretion (ADME), were evaluated for the selected ligands using the SwissADME online tool (http://www.swissadme.ch/index.php). This analysis is critical for predicting the in vivo pharmacokinetic behavior of compounds, offering valuable insights into their potential as drug candidates. Assessing these parameters provides a clearer understanding of how a compound may be absorbed, distributed throughout the body, metabolized by enzymes, and ultimately excreted. The results from this in silico ADME analysis are essential for guiding drug research and development, enabling the identification of compounds with favorable pharmacokinetic profiles that are more likely to succeed in subsequent stages of drug development.

Bioactivity score and bioavailability radar

The bioactivity scores for the ligands were evaluated using the Molinspiration online platform (http://www.molinspiration.com/). This process involved utilizing the canonical SMILES representations of the ligands, sourced from PubChem. The analysis focused on several properties, including interactions with G-protein coupled receptors (GPCRs), enzyme inhibitors (EIs), kinase inhibitors (KIs), nuclear receptor ligands (NRLs), and ion channel modulators (ICMs). Additionally, the bioavailability radar of each ligand was assessed using the SwissADME tool (http://www.swissadme.ch/index.php), which provides a rapid evaluation of the likelihood of a compound being orally bioavailable.

Results

Weight Variation Test

The weight variation test was conducted on a sample of 20 chewable lozenges to ensure dosage consistency and accuracy. Each tablet was individually weighed, and the results were recorded. The average weight of the tablets was calculated to be 0.516, with an upper limit of 0.5414 and a lower limit of 0.490, based on a permissible variation of $\pm 5\%$ as per Indian Pharmacopoeia. Out of the 20 tablets tested, 18 passed the weight variation test, while 2 tablets failed to meet the specified limits. This quality control measure ensures uniformity in tablet weight, contributing to the reliability and effectiveness of the medication. The summarized results of the weight variation test are presented in Table 2.

 Table (2): Weight variation test for the formulated lozenges, showing the individual weights of 20 lozenges to assess consistency and uniformity in the manufacturing process.

SI No	Weight	SI No	Weight
1.	0.540	11.	0.512
2.	0.491	12.	0.491
3.	0.591	13.	0.522
4.	0.534	14.	0.541
5.	0.519	15.	0.522
6.	0.510	16.	0.541
7.	0.548	17.	0.542
8.	0.522	18.	0.498
9.	0.468	19.	0.526
10.	0.504	20	0.540

Mean Weight: 0.5231 g Standard Deviation: 0.0265 g Range of Weights: 0.468 g to 0.591 g

Number of Tablets That Failed the Test: 2

Friability Test

The friability test, a crucial aspect of pharmaceutical quality control, assessed the resistance of tablets to mechanical stress during handling, transportation, and use. The experimental setup, including the friability testing apparatus, was meticulously prepared and calibrated in accordance with standard procedures. A carefully selected sample of 20 tablets, ensuring uniformity in size, shape, and coating, underwent dedusting if necessary. The tablets' initial weight was accurately measured, and they were subjected to 100 rotations in the friability testing drum as per pharmacopeial standards. After testing, loose particles were removed, and a final weight measurement was taken. The calculated percentage friability, [(Initial Weight - Final Weight) / Initial Weight] × 100, was 0.79%. The obtained friability percentage of 0.79% for the chewable lozenge was compared to the pharmacopeial monograph limits, which specify a threshold below 1%, confirming the compliance of the tablets with the specified criteria. The entire procedure adhered strictly to current Good Manufacturing Practice (cGMP) guidelines and pharmacopeial standards, ensuring the tablets' quality and durability in real-world conditions.

Hardness Test

The hardness of the polyherbal lozenges was measured at 5.8 ± 0.4 kg/cm², falling within the acceptable range for chewable formulations. This ensures adequate compactness to maintain structural integrity while allowing controlled dissolution. The results confirm that the formulation meets the physical strength criteria specified by Indian Pharmacopoeia (IP) standards for chewable tablets.

Moisture Content Test

The moisture content, expressed as a percentage by weight, was found to range from 1.70% w/w to 1.95% w/w, indicating that the water content of the sample falls within acceptable limits. The determination was performed using the Karl Fischer titration method, specifically the volumetric (direct titration) technique, which is suitable for measuring moisture content in solid and semi-solid pharmaceutical formulations.

Dissolution Test

The dissolution testing protocol for chewable lozenges was designed to determine the percentage of drug release under simulated physiological conditions. Using USP Dissolution Apparatus II (Paddle) with a pH 5.8 artificial saliva solution as the dissolution medium, the temperature was maintained at $37 \pm 0.5^{\circ}$ C. Calibration was conducted in accordance with manufacturer guidelines to ensure proper paddle rotation speed.

For sample preparation, a representative number of lozenges were selected, and individual weights were recorded. Microsoft Excel was used to calculate the percentage of drug release. Dissolution vessels, each containing one lozenge and 900 mL of pH 5.8 artificial saliva buffer, were utilized. The dissolution process commenced at a predetermined paddle rotation speed, with samples collected at 5, 10, 15, 20, and 25 minutes. Withdrawn samples were replaced with an equal volume of fresh dissolution medium, filtered through filter paper, and analyzed.

The results demonstrated cumulative drug release of 0.282% at 5 minutes, 17.84% at 10 minutes, 45.92% at 15 minutes, 75.68% at 20 minutes, and 91.26% at 25 minutes, providing a comprehensive evaluation of drug release under controlled conditions. The dissolution study is summarized in Table 3, and the standard curve is illustrated in Fig. 6.

Culture Sensitivity Test

In the culture sensitivity test, the polyherbal chewable lozenges were evaluated for their antimicrobial efficacy against S. aureus and E. coli. Bacterial cultures were inoculated onto nutrient agar plates, and the lozenges were applied. Incubation was conducted at 37°C for 24 hours, after which the zones of inhibition were measured, revealing a diameter of 15 mm for S. aureus and 25 mm for E. coli. These results demonstrated significant antimicrobial activity of the polyherbal lozenges against both bacterial strains, with E. coli exhibiting greater susceptibility. Further investigation is warranted to elucidate the specific mechanisms underlying this antimicrobial effect and to explore the potential therapeutic applications of these polyherbal lozenges in treating mouth and throat infections. When compared to Azithromycin, which typically exhibits inhibition zones ranging from 13–22 mm for S. aureus and 12–20 mm for E. coli, the polyherbal lozenges demonstrated comparable efficacy against S. aureus and superior inhibition against E. coli. These findings suggest that the polyherbal lozenges possess promising antimicrobial potential, potentially matching the activity of Azithromycin in targeting oral pathogens. Further studies are necessary to explore their clinical applications.

Culture sensitivity test strains are illustrated in Figure 5, and a summary of all results is presented in Table 4.



Figure (5): Culture sensitivity test results against (A) Escherichia coli and (B) Staphylococcus aureus. The images demonstrate the effectiveness of the tested agents in inhibiting bacterial growth, as indicated by the zones of inhibition surrounding the discs.

Table (3): Dissolution	profile of the	formulated herbal	lozenges, s	howing dru	g release	over time
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Time	Absorbance	concentration	concdil.Fac	connc in 900	cumulative amou exc	conc in mg	%Drug released
5	0.002	0.015748031	0.157480315	141.7322835	141.73	0.14173	0.282
10	0.124	0.976377953	9.763779528	8787.401575	8929.131575	8.929131575	17.84
15	0.198	1.559055118	15.59055118	14031.49606	22960.62764	22.96062764	45.92
20	0.21	1.653543307	16.53543307	14881.88976	37842.5174	37.8425174	75.68
25	0.11	0.866141732	8.661417323	7795.275591	45637.79299	45.63779299	91.26



Figure (6): Standard curve with the equation y=0.127x+5E-06 y=0.127x+5E-06 and R2=1R2=1, showing a linear relationship between concentration and absorbance.

Table (4): Summa	ry of herba	l lozenge analysis:	: weight, friability,	moisture, dissolution,	and antimicrobial activity results.
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Test	Description	Results
Weight Variation	Ensured dosage consistency and accuracy by weighing 20 lozenges individually. Average weight: 0.516, within permissible variation of ±5%.	18 passed, 2 failed to meet weight limits.
Friability	Tested tablets' resistance to mechanical stress. 10 tablets underwent 100 rotations; friability: 0.79%, below the acceptable limit (<1%).	Tablets complied with pharmacopeial standards.
Moisture content	Moisture content ranged from 1.70% to 1.95% w/w, complying with acceptable limits.	Within acceptable limits.
Dissolution	Developed dissolution protocol using USP Dissolution Apparatus II (Paddle). Cumulative drug release ranged from 0.282% to 91.26% over 25 minutes.	Provided comprehensive evaluation of drug release under controlled conditions and the drug release was in acceptable range.
Culture Sensitivity	Assessed antimicrobial efficacy against S. aureus and E. coli. Significant activity observed; zones of inhibition: 15 mm (S. aureus), 25 mm (E. coli).	Demonstrated significant antimicrobial activity against tested bacterial strains.

Docking Analysis

The docking study revealed that among the phytochemicals from Karpuravalli, Ginger, Cardamom, and Clove Oil, several exhibited promising interactions with the Dihydrofolate Reductase (DHFR) enzyme from Staphylococcus aureus type S1. Rosmarinic Acid from Karpuravalli demonstrated the highest binding affinity, with a binding energy of -6.9 kcal/mol, forming three hydrogen bonds with THR 63, ASN 64, and THR 96. Additionally, it exhibited a low inhibition constant of $0.87 \times 10-5$

 μ M, indicating strong potential as an inhibitor. Beta-Sitosterol from Cardamom also displayed a significant binding energy of -7.2 kcal/mol; however, it did not form any hydrogen bonds, suggesting that non-polar interactions might primarily govern its binding. Gingerol from Ginger exhibited a binding energy of -6.0 kcal/mol, forming two hydrogen bonds with GLN 33 and SER 135, while Zingerone showed a comparable binding affinity, interacting with ASN 145 and ARG 157. These findings highlight the potential of these phytochemicals, particularly those forming

hydrogen bonds, as DHFR inhibitors. The detailed results are summarized in Table 5, while Figure 7 presents the 3D docked illustrations, and Figure 8 illustrates the 2D interactions.

The docking analysis of phytochemicals from Karpuravalli, Ginger, Cardamom, and Clove Oil against the UDP-N-Acetylglucosamine Enolpyruvyl Transferase enzyme (PDB ID: 1UAE) from Escherichia coli revealed significant variations in binding affinities and interactions. Rosmarinic Acid from Karpuravalli demonstrated the strongest binding affinity, with a binding energy of -8.7 kcal/mol and an exceptionally low inhibition constant of $0.04 \times 10-5$ M. It formed seven hydrogen bonds with GLY 164, VAL 163, GLU 188, ARG 232, ASP 305, ASN 23, and LYS 22, indicating a highly stable and specific interaction. Gingerol from Ginger also exhibited strong binding, with a binding energy of -7.0 kcal/mol, forming three hydrogen bonds with GLY 164, VAL 163, and GLU 188. Paradol and Zerumbone from Ginger displayed binding energies of -6.6 kcal/mol and -6.7 kcal/mol, respectively, with Paradol forming five hydrogen bonds, whereas Zerumbone formed none, suggesting that hydrogen bonding is not the sole determinant of binding affinity. Beta-Sitosterol from Cardamom also demonstrated a notable binding energy of -6.6 kcal/mol, without forming hydrogen bonds, similar to β-Caryophyllene from Clove Oil, which exhibited the same binding energy with no hydrogen bonding. Other phytochemicals, such as Carvacrol and Eugenol, formed single hydrogen bonds with moderate binding energies of -5.7 kcal/mol and -5.5 kcal/mol, respectively, interacting with amino acids such as THR

304 and ARG 232. These findings suggest that phytochemicals forming multiple hydrogen bonds, such as Rosmarinic Acid and Paradol, are more likely to exhibit strong interactions with the target enzyme, potentially contributing to their inhibitory effects against E. coli. This insight could be valuable in the development of novel antimicrobial agents. The detailed results are presented in Table 7, with 3D docked illustrations in Figure 9 and 2D interactions in Figure 10.

Comparison of Binding energies between Ligands and Targeted Protein S1 DHFR dihydrofolate reductase enzyme from Staphylococcus aureus type S1 with Standard Antibiotic

Trimethoprim

Binding Energy: Approximately -8.0 to -9.0 kcal/mol.

Trimethoprim is a well-known DHFR inhibitor used to treat bacterial infections. Its high binding affinity reflects its strong inhibitory effect on bacterial DHFR.[63]

Beta-Sitosterol

Binding Energy: -7.2 kcal/mol.

This is comparable to Trimethoprim (-8.0 to -9.0 kcal/mol), indicating that Beta-Sitosterol has the potential to be a highly effective DHFR inhibitor. Although it does not form hydrogen bonds, its strong binding energy suggests that non-polar interactions dominate its binding.

Rosmarinic Acid

Binding Energy: -6.9 kcal/mol. This is slightly weaker than Trimethoprim but still indicates significant potential as a DHFR inhibitor. It forms three hydrogen bonds with THR 63, ASN 64, and THR 96, which are critical for stabilizing the interaction. [64]

Other Phytochemicals

Compounds like Gingerol, Zingerone, and Zerumbone have binding energies in the range of -6.0 to -6.1 kcal/mol, which are weaker than Trimethoprim but still indicate moderate potential as inhibitors. Phytochemicals with binding energies below -6.0 kcal/mol (e.g., Caffeic Acid, Carvacrol, Thymol) may still contribute to antimicrobial activity through synergistic effects or alternative mechanisms, such as disrupting bacterial membranes or biofilm formation.

The binding energy values of Beta-Sitosterol and Rosmarinic Acid are particularly promising, as they are comparable to or slightly weaker than Trimethoprim, a standard antibiotic targeting DHFR. These phytochemicals, especially those forming hydrogen bonds, have the potential to be developed into novel antimicrobial agents. However, binding energy alone does not guarantee efficacy, as factors like bioavailability, bacterial resistance, and pharmacokinetics must also be considered. Further in vitro and in vivo studies are necessary to validate their antimicrobial potential. Comparison results are summarised in Table 6

Comparison of Binding energies between Ligand and Targeted Protein Udp-N-Acetylglucosamine Enolpyruvyl Transferase of E coli with Standard Antibiotics

Fosfomycin

Binding Energy: Approximately -7.5 to -8.5 kcal/mol. Fosfomycin is a broad-spectrum antibiotic that irreversibly inhibits MurA, a key enzyme in bacterial cell wall synthesis.[65]

Rosmarinic Acid

Binding Energy: -8.7 kcal/mol. This is stronger than Fosfomycin (-7.5 to -8.5 kcal/mol), indicating that Rosmarinic Acid has the potential to be a highly effective MurA inhibitor. The formation of seven hydrogen bonds with key amino acids (GLY 164, VAL 163, GLU 188, ARG 232, ASP 305, ASN 23, and LYS 22) further supports its strong binding affinity.

Gingerol

Binding Energy: -7.0 kcal/mol. This is comparable to Fosfomycin, suggesting that Gingerol could also be a potent MurA inhibitor. It forms three hydrogen bonds with GLY 164, VAL 163, and GLU 188, which are critical for stabilizing the interaction.

Other Phytochemicals

Compounds like Zerumbone, Paradol, Beta-Sitosterol, and β -Caryophyllene have binding energies in the range of -6.6 to -6.7 kcal/mol, which are slightly weaker than Fosfomycin but still indicate significant potential as inhibitors. Phytochemicals with binding energies below -6.5 kcal/mol (e.g., Caffeic Acid, Carvacrol, Thymol) may still contribute to antimicrobial activity through synergistic effects or alternative mechanisms, such as disrupting bacterial membranes or biofilm formation.

The binding energy values of Rosmarinic Acid and Gingerol are particularly promising, as they are comparable to or even stronger than Fosfomycin, a standard antibiotic targeting MurA. These phytochemicals, especially those forming multiple hydrogen bonds, have the potential to be developed into novel antimicrobial agents. However, binding energy alone does not guarantee efficacy, as factors like bioavailability, bacterial resistance, and pharmacokinetics must also be considered. Further in vitro and in vivo studies are necessary to validate their antimicrobial potential. The comparison results are summarised in Table 8

Table (5): Binding Parameters between Ligands and Targeted Protein S1 DHFR dihydrofolate reductase enzyme from Staphylococcus aureus type S1.

Phytochemicals	Binding Energy (Kcal/mol)	Inhibition constant × 10-5 µM	Number of hydrogen bonds	Amino Acids involved in hydrogen bonding
	, ,	Karpuravalli		, , , , ,
Carvacrol	-5.5	9.25	0	-
Rosmarinic Acid	-6.9	0.87	3	THR 63, ASN 64, THR 96
Caffeic acid	-5.8	5.57	3	LYS 144, ASN 145, ASN 26
Thymol	-5.3	12.96	1	ASN 26
	•	Ginger		
Gingerol	-6.0	3.97	2	GLN 33, SER 135
Paradol	-5.9	4.71	3	LYS 29, GLN 33, SER 135
Zingerone	-6.0	3.97	2	ASN 145, ARG 157
Zerumbone	-6.0	3.97	0	-
Shogaol	-5.8	5.57	3	ASN 64, THR 96, LEU 97
		Cardamom		
Beta Sitosterol	-7.2	0.52	0	-
α-Terpinyl acetate	-5.9	4.71	1	LYS 144
Sabinene	-5.5	9.25	0	-
Linalool	-4.9	25.47		ASN 26
α-Pinene	-5.5	9.25	0	-
		Clove Oil		
Eugenol	-5.3	12.96	1	ARG 157
β-Caryophyllene	-6.1	3.36	0	-

Table (6): The binding energies of the phytochemicals are compared with Trimethoprim and other DHFR inhibitors to assess their potential as antimicrobial agents.

Phytochemicals	Binding Energy (Kcal/mol)	Comparison with Trimethoprim
Beta-Sitosterol	-7.2	Comparable to Trimethoprim
Rosmarinic Acid	-6.9	Slightly weaker
Gingerol	-6.0	
Zingerone	-6.0	
Zerumbone	-6.0	
β-Caryophyllene	-6.1	
Paradol	-5.9	
Caffeic Acid	-5.8	
Shogaol	-5.8	Weaker
Carvacrol	-5.5	
Sabinene	-5.5	
α-Pinene	-5.5	
Thymol	-5.3	
Eugenol	-5.3	
α-Terpinyl Acetate	-5.9	
Linalool	-4.9	
Paradol Caffeic Acid Shogaol Carvacrol Sabinene α-Pinene Thymol Eugenol α-Terpinyl Acetate Linalool	-5.9 -5.8 -5.8 -5.5 -5.5 -5.5 -5.5 -5.3 -5.3 -5.9 -4.9	Weaker

Table (7): Binding Parameters between Ligand and Targeted Protein Udp-N-Acetylglucosamine Enolpyruvyl Transferase of E coli.

Phytochemicals	Binding Energy (Kcal/mol)	Inhibition constant	Number of hydrogen bonds	Amino Acids involved in hydrogen bonding						
Karpuravalli										
Carvacrol	-5.7	1	THR 304							
Rosmarinic Acid	-8.7	0.04	7	GLY 164, VAL 163, GLU 188, ARG 232, ASP 305, ASN 23, LYS 22						
Caffeic acid	-6.3	2.39	3	VAL 161, ARG 232, ASP 305						
Thymol	-5.6									
		Gir	nger							
Gingerol	-7.0	0.73	3	GLY 164, VAL 163, GLU 188						
Paradol	-6.6	1.44	5	GLY 164, VAL 163, ARG 232, GLU 190, ASN 23						
Zingerone	-6.1	3.36	3	GLY 164, VAL 163, GLU 188						
Zerumbone	-6.7	1.22	0	-						
Shogaol	-6.5		3	GLY 164, VAL 163, ARG 232						
		Card	amom							
Beta Sitosterol	-6.6	1.44	0	-						
α-Terpinyl acetate	-6.2	2.84	3	ASN 23, LYS 22, ARG 120						
Sabinene	-5.4	10.95	0	-						
Linalool	-5.2	15.35	1	SER 245						
α-Pinene	-5.2	15.35	0	-						
		Clov	/e Oil							
Eugenol	-5.5	9.25	1	ARG 232						
β-Caryophyllene	-6.6	1.44	0	-						

Table (8): The binding energies of the phytochemicals are compared with Fosfomycin and other MurA inhibitors to assess their potential as antimicrobial agents.

Phytochemicals	Binding Energy (Kcal/mol)	Comparison with Fosfomycin
Rosmarinic Acid	-8.7	Stronger than Fosfomycin
Gingerol	-7.0	Comparable to Fosfomycin
Zerumbone	-6.7	
Paradol	-6.6	Slightly wooker
Beta-Sitosterol	-6.6	Slightly weaker
β-Caryophyllene	-6.6	
Caffeic Acid	-6.3	
α-Terpinyl Acetate	-6.2	Weaker
Zingerone	-6.1	

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Shogaol	-6.5
Carvacrol	-5.7
Thymol	-5.6
Eugenol	-5.5
Sabinene	-5.4
Linalool	-5.2
α-Pinene	-5.2







Figure (7): Illustration of 3D Docked poses of staphylococcus aureus showing binding site (A)Carvacrol, (B)Rosmarinic acid, (c)Caffeic acid, (D)Thymol, (E)Gingerol, (F)Paradol, (G)Zingerone, (H)Zerumbone, (I)Shogaol, (J)Beta Sitosterol, (K)α-Terpinyl acetate, (L)Sabinene, (M)Linalool, (N)α-Pinene, (O)Eugenol, (P)β-Caryophyllene.



Figure (8): 2D Interaction representation of ligand and receptor protein of Staphylococcus aureus (A)Carvacrol, (B)Rosmarinic acid, (c)Caffeic acid, (D)Thymol, (E)Gingerol, (F)Paradol, (G)Zingerone, (H)Zerumbone, (I)Shogaol, (J)Beta Sitosterol, (K)α-Terpinyl acetate, (L)Sabinene, (M)Linalool, (N)α-Pinene, (O)Eugenol, (P)β-Caryophyllene.







Figure (9): Illustration of 3D Docked poses of E coli showing binding site(A)Carvacrol, (B)Rosmarinic acid, (c)Caffeic acid, (D)Thymol, (E)Gingerol, (F)Paradol, (G)Zingerone, (H)Zerumbone, (I)Shogaol, (J)Beta Sitosterol, (K)α-Terpinyl acetate, (L)Sabinene, (M)Linalool, (N)α-Pinene, (O)Eugenol, (P)β-Caryophyllene.



Figure (10): 2D interaction representation of ligand and receptor protein of E. coli (A)Carvacrol, (B)Rosmarinic acid, (c)Caffeic acid, (D)Thymol, (E)Gingerol, (F)Paradol, (G)Zingerone, (H)Zerumbone, (I)Shogaol, (J)Beta Sitosterol, (K)α-Terpinyl acetate, (L)Sabinene, (M)Linalool, (N)α-Pinene, (O)Eugenol, (P)β-Caryophyllene.

Pharmacokinetic and Drug Likeness Screening of Phytochemicals

Drug properties of the 16 selected phytochemicals were assessed using Lipinski's rule of five and ADME analysis. The evaluation covered molecular weight, hydrogen bond donors, hydrogen bond acceptors, lipophilicity (LogP), and molar refractivity. Phytochemicals including Carvacrol, Thymol, Gingerol, Paradol, Zingerone, Zerumbone, Shogaol, α -Terpinyl acetate, Sabinene, Linalool, α -Pinene, Eugenol, and β -Caryophyllene met all five criteria of Lipinski's rule. However, Rosmarinic Acid, Caffeic Acid, and β -Sitosterol showed deviations in one or two criteria, which are deemed acceptable. These results are shown in Table 9.

The violations of Lipinski's rule of five, such as high molecular weight and excessive lipophilicity, significantly impact

the bioavailability and pharmacokinetics of compounds like β-Sitosterol and Rosmarinic Acid. High molecular weight and excessive hydrogen bonding in Rosmarinic Acid lead to poor solubility and limited gastrointestinal absorption, reducing its bioavailability. Similarly, the extreme lipophilicity of β-Sitosterol results in low aqueous solubility, hindering its absorption and distribution. Furthermore, high lipophilicity can cause sequestration in fatty tissues, reducing the compound's availability at the target site and altering its pharmacokinetic profile. These violations collectively restrict the therapeutic efficacy of these compounds, as their ability to reach effective concentrations in systemic circulation and target tissues is compromised. Addressing these issues through formulation strategies or structural modifications is essential to improve their bioavailability and pharmacokinetic properties for therapeutic applications.

The ADME (Absorption, Distribution, Metabolism, and Excretion) screening of phytochemicals derived from Karpuravalli, Ginger, Cardamom, and Clove Oil using the SwissADME tool revealed detailed insights into their pharmacokinetic profiles and drug-likeness. Carvacrol, a phytochemical from Karpuravalli, exhibited good solubility, high gastrointestinal absorption (GIA), and the ability to cross the blood-brain barrier (BBB). It also demonstrated inhibition of CYP1A2 but was neither a P-glycoprotein (P-gp) substrate nor a CYP3A4 inhibitor, resulting in a favorable bioavailability score of 0.55. In contrast, Rosmarinic Acid showed poor solubility, low GIA, and no BBB permeability. It was not a P-gp substrate or a CYP enzyme inhibitor but maintained a comparable bioavailability score of 0.56.

Phytochemicals from Ginger, including Gingerol, Paradol, and Shogaol, displayed high GIA, BBB permeability, and CYP1A2 inhibition. These compounds exhibited moderate to high lipophilicity, with iLOGP values ranging from 2.09 to 3.65, and consistent bioavailability scores of 0.55. On the other hand, Beta-Sitosterol from Cardamom demonstrated low solubility, low GIA, and no BBB permeability or CYP enzyme inhibition, despite its high lipophilicity (iLOGP of 5.05) and a standard bioavailability score.

Eugenol from Clove Oil also showed high GIA, BBB permeability, and CYP1A2 inhibition, reflecting a well-balanced pharmacokinetic profile with an iLOGP of 2.37 and a bioavailability score of 0.55. These findings indicate that while several phytochemicals possess favorable pharmacokinetic properties, their potential as drug candidates varies significantly, particularly in terms of solubility, GIA, and enzyme inhibition. These results are summarized in Table 10.

To improve the solubility and absorption of phytochemicals like Rosmarinic Acid and β -Sitosterol, several formulation and chemical modification strategies can be employed. Nanoparticle encapsulation, such as using liposomes or polymeric nanoparticles, can enhance the solubility and bioavailability of poorly soluble compounds like β -Sitosterol by improving their dispersion in aqueous environments. Solid dispersion

techniques, where the compound is mixed with hydrophilic carriers like polyethylene glycol, can increase the dissolution rate and solubility of Rosmarinic Acid. Cyclodextrin complexation offers another approach by forming inclusion complexes that enhance solubility and stability. Additionally, chemical modifications, such as prodrug design, can optimize lipophilicity or reduce hydrogen bonding, improving absorption. Coadministration with permeation enhancers, such as sodium caprate, or bioavailability boosters, like piperine, can further enhance gastrointestinal absorption. These strategies, supported by experimental validation, can address solubility and absorption challenges, thereby improving the therapeutic potential of these phytochemicals.

Bioavailability Radar

The radar plots provided illustrate the pharmacokinetic profiles of various phytochemicals, emphasizing their drug-like properties. Several compounds, as depicted in Plots 1, 3, 7, 11, and 14, exhibit high lipophilicity (LIPO) and insaturation (INSATU), with lower polarity (POLAR) and solubility (INSOLU). This indicates that these compounds are highly lipophilic and less soluble, which may impact their absorption and distribution.

Conversely, Plots 2, 5, 6, 8, 9, 10, 12, and 13 display more balanced profiles, characterized by moderate peaks in lipophilicity and flexibility (FLEX). These properties suggest a more even distribution of pharmacokinetic attributes, potentially contributing to improved drug-likeness. Notably, certain compounds, particularly those in Plots 10 and 13, exhibit higher polarity and size, indicating a larger and more polar molecular structure, which may influence their bioavailability and permeability.

Overall, the radar plots provide valuable insights into the varying degrees of lipophilicity, flexibility, solubility, and polarity among the phytochemicals, highlighting their potential effectiveness as drug candidates. These profiles can serve as a guide for further selection and optimization of these compounds for therapeutic applications. The bioavailability radars are illustrated in Fig. 10.

Table (9): Lipinski's Rule of	of 5 a	analysis for phytod	chem	icals in	Karpura	avalli,	Ginger,	Cardamom,	and Clove Oil,	including mass,	hydrogen l	oond donors	acceptors,
LOGP, and molar refractivi	ity.												

Phytochemicals	Mass	Hydrogen bond donor	Hydrogen bond acceptor	LOGP	Molar refractivity				
Karpuravalli									
Carvacrol	150	1	1	2.82	46.93				
Rosmarinic Acid	360	5	8	1.76	89.79				
Caffeic acid	180	3	4	1.19	46.44				
Thymol	150	1	1	2.82	46.93				
Ginger									
Gingerol	294	2	4	3.23	82.75				
Paradol	278	1	3	4.26	81.36				
Zingerone	194	1	3	1.92	53.66				
Zerumbone	218	0	1	4.21	69.29				
Shogaol	276	1	3	4.03	81.26				
		Cardam	nom						
Beta Sitosterol	414	1	1	8.02	128.21				
α-Terpinyl acetate	196	0	2	3.07	56.94				
Sabinene	136	0	0	2.99	43.75				
Linalool	154	1	1	2.66	49.48				
α-Pinene	136	0	0	2.99	43.75				
Clove Oil									
Eugenol	164	1	2	2.12	48.55				
β-Caryophyllene	204	0	0	4.72	66.74				

Table (10): In silico pharmacokinetic properties of phytochemicals using Swiss ADME, including solubility, gastrointestinal absorption, BBB permeation, P-gp substrate potential, CYP inhibition, and bioavailability scores.

Phytochemicals	ESOL (Log S)	GIA	BBB P-gp substrate		CYP3A4 inhibitor	CYP1A2 inhibitor	(iLOGP)	Bioavailability score		
Karpuravalli										
Carvacrol	-3.31	High	Yes	No	No	Yes	2.24	0.55		

Rosmarinic Acid	-3.44	Low	No	No	No	No	1.48	0.56		
Caffeic acid	-1.89	High	No	No	No	No	0.97	0.56		
Thymol	-3.19	High	Yes	No	No	Yes	2.32	0.55		
	Ginger									
Gingerol	-2.96	High	Yes	No	No	Yes	3.48	0.55		
Paradol	-3.72	High	Yes	No	No	Yes	3.65	0.55		
Zingerone	-1.80	High	Yes	No	No	Yes	2.09	0.55		
Zerumbone	-3.68	High	Yes	No	No	No	2.78	0.55		
Shogaol	-3.70	High	Yes	No	No	Yes	3.28	0.55		
				Cardamom						
Beta Sitosterol	-7.90	Low	No	No	No	No	5.05	0.55		
α-Terpinyl acetate	-3.35	High	Yes	No	No	No	2.93	0.55		
Sabinene	-2.57	Low	Yes	No	No	No	2.65	0.55		
Linalool	-2.40	High	Yes	No	No	No	2.70	0.55		
α-Pinene	-3.51	Low	Yes	No	No	No	2.63	0.55		
Clove Oil										
Eugenol	-2.46	High	Yes	No	No	Yes	2.37	0.55		
β-Caryophyllene	-3.87	Low	No	No	No	No	3.25	0.55		







Figure (11): Bioavailability Raders (A)Carvacrol, (B)Rosmarinic acid, (c)Caffeic acid, (D)Thymol, (E)Gingerol, (F)Paradol, (G)Zingerone, (H)Zerumbone, (I)Shogaol, (J)Beta Sitosterol, (K)α-Terpinyl acetate, (L)Sabinene, (M)Linalool, (N)α-Pinene, (O)Eugenol, (P)β-Caryophyllene.

INSOLU

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Bioactivity Score

The bioactivity scores of various phytochemicals from Karpuravalli, Ginger, Cardamom, and Clove Oil, evaluated using Molinspiration.com, provide detailed insights into their potential interactions and efficacy. Karpuravalli Phytochemicals: Carvacrol exhibits a consistently negative bioactivity profile across most parameters, with scores of -1.02 for GPCR, -0.51 for ICM, -1.15 for KI, -0.70 for NRL, -1.25 for PI, and -0.56 for EI, suggesting strong biological activity. Rosmarinic Acid presents a more balanced profile with values of 0.17 for GPCR, -0.08 for ICM, -0.18 for KI, 0.57 for NRL, and 0.15 for PI, with an average score of 0.24, indicating moderate biological effects. Caffeic Acid demonstrates negative bioactivity in most parameters, with scores of -0.48 for GPCR, -0.23 for ICM, -0.81 for KI, -0.10 for NRL, -0.79 for PI, and -0.09 for EI, suggesting moderate to strong biological interactions. Thymol shows a negative bioactivity profile similar to Carvacrol, with scores of -1.05 for GPCR, -0.53 for ICM, -1.29 for KI, -0.78 for NRL, -1.34 for PI, and -0.57 for EI, indicating strong interaction potential. Ginger Phytochemicals: Gingerol displays positive scores for GPCR (0.16) and NRL (0.20), with a higher positive value for EI (0.38), suggesting moderate biological activity. However, it has negative scores for KI (-0.33), ICM (0.04), and PI (0.15), indicating a varied interaction profile. Paradol exhibits mixed scores, with -0.01 for GPCR, -0.04 for ICM, -0.47 for KI, 0.08 for NRL, -0.09 for PI, and 0.18 for EI, suggesting moderate biological activity. Zingerone presents a negative profile with scores of -0.58 for GPCR, -0.18 for ICM, -1.15 for KI, -0.59 for NRL, -0.72 for PI, and -0.07 for EI, indicating significant interaction potential. Zerumbone has mixed bioactivity scores, with values of -0.28 for GPCR, -0.08 for ICM, -1.07 for KI, 0.22 for NRL, -0.52 for PI, and 0.24 for EI, suggesting moderate to strong biological interactions. Shogaol

shows positive scores for GPCR (0.06), NRL (0.20), and EI (0.29), while being slightly negative for KI (-0.50) and PI (-0.05), indicating moderate biological activity. Cardamom Phytochemicals: Beta-Sitosterol exhibits positive scores across various parameters, with values of 0.14 for GPCR, 0.04 for ICM, -0.51 for KI, 0.73 for NRL, 0.07 for PI, and 0.51 for EI, suggesting strong biological activity potential. α-Terpinyl Acetate shows a negative score for KI (-1.14) and GPCR (-0.35), with positive values for ICM (0.08), NRL (0.00), PI (-0.50), and EI (0.28), suggesting a varied interaction profile. Sabinene has a predominantly negative bioactivity profile, with scores of -1.15 for GPCR, -0.33 for ICM, -1.79 for KI, -0.69 for NRL, -0.78 for PI, and -0.60 for EI, indicating strong biological activity. Linalool exhibits a generally negative interaction profile, with scores of -0.73 for GPCR, 0.07 for ICM, -1.26 for KI, -0.06 for NRL, -0.94 for PI, and 0.07 for EI, suggesting moderate to high biological interaction potential. α-Pinene shows negative bioactivity with scores of -0.48 for GPCR, -0.43 for ICM, -1.50 for KI, -0.62 for NRL, -0.85 for PI, and -0.34 for EI, indicating strong interaction potential. Clove Oil Phytochemicals: Eugenol presents a strong negative profile, with scores of -0.86 for GPCR, -0.36 for ICM, -1.14 for KI, -0.78 for NRL, -1.29 for PI, and -0.41 for EI, indicating significant biological interactions. β-Caryophyllene exhibits mixed bioactivity, with negative scores of -0.34 for GPCR, -0.78 for KI, and -0.60 for PI, but positive values of 0.28 for ICM, 0.13 for NRL, and 0.19 for EI, suggesting varied interaction potential. Overall, the phytochemicals from Karpuravalli, Ginger, Cardamom, and Clove Oil exhibit diverse bioactivity profiles, with several compounds showing strong interaction potentials, while others present more moderate or varied interactions. These findings provide valuable insights for further investigation into their potential therapeutic applications (Table 11).

 Table (11): Bioactivity scores of phytochemicals using Molinspiration.com, evaluating GPCR, ion channel modulation, kinase inhibition, nuclear receptor ligand, protease inhibition, and enzyme inhibition activities.

Phytochemicals	GPCR	ICM	КІ	NRL	PI	EI					
Karpuravalli											
Carvacrol	-1.02	-0.51	-1.15	-0.70	-1.25	-0.56					
Rosmarinic Acid	0.17	-0.08	-0.18	0.57	0.15	0.24					
Caffeic acid	-0.48	-0.23	-0.81	-0.10	-0.79	-0.09					
Thymol	-1.05	-0.53	-1.29	-0.78	-1.34	-0.57					
		Ginger									
Gingerol	0.16	0.04	-0.33	0.20	0.15	0.38					
Paradol	-0.01	-0.04	-0.47	0.08	-0.09	0.18					
Zingerone	-0.58	-0.18	-1.15	-0.59	-0.72	-0.07					
Zerumbone	-0.28	-0.08	-1.07	0.22	-0.52	0.24					
Shogaol	0.06	0.01	-0.50	0.20	-0.05	0.29					
Cardamom											
Beta Sitosterol	0.14	0.04	-0.51	0.73	0.07	0.51					
α-Terpinyl acetate	-0.35	0.08	-1.14	0.00	-0.50	0.28					
Sabinene	-1.15	-0.33	-1.79	-0.69	-0.78	-0.60					
Linalool	-0.73	0.07	-1.26	-0.06	-0.94	0.07					
α-Pinene	-0.48	-0.43	-1.50	-0.62	-0.85	-0.34					
Clove Oil											
Euginol	-0.86	-0.36	-1.14	-0.78	-1.29	-0.41					
β-Caryophyllene	-0.34	0.28	-0.78	0.13	-0.60	0.19					

Discussion

The study assessed the quality, efficacy, and potential therapeutic benefits of polyherbal chewable lozenges by evaluating key pharmaceutical and biological attributes, including weight variation, friability, moisture content, dissolution, antimicrobial activity, and phytochemical interactions. Quality assessments confirmed that 90% of the lozenges met dosage consistency standards, with minor deviations noted. The friability test demonstrated high mechanical resistance (0.79%), ensuring durability, while moisture content remained within acceptable limits (1.70% to 1.95%), indicating stability. The dissolution test showed an effective drug release profile, with 91.26% release at 25 minutes, supporting efficient bioavailability under simulated

physiological conditions. The antimicrobial evaluation demonstrated significant efficacy against S. aureus and E. coli, with E. coli exhibiting greater susceptibility. These findings align with prior studies emphasizing the antibacterial properties of phytochemicals in oral formulations. Molecular docking studies identified Rosmarinic Acid (Karpuravalli) and Beta-Sitosterol (Cardamom) as key bioactive components, exhibiting high binding affinities with bacterial enzymes, suggesting potential inhibitory mechanisms. Pharmacokinetic screening, based on Lipinski's Rule of Five, revealed varied drug-like properties, indicating that while some compounds showed optimal bioavailability, others may require formulation enhancement. Bioactivity scores further highlighted the antimicrobial potential, with select compounds exhibiting strong interactions with bacterial targets. This study underscores the adherence of polyherbal lozenges to pharmaceutical quality standards and their potential effectiveness as antimicrobial agents. The integration of in silico and in vitro methodologies enhances the reliability of these findings. Future research should focus on optimizing formulation strategies to improve pharmacokinetic profiles, conducting detailed mechanistic studies, and validating efficacy through comprehensive clinical trials. Establishing pharmacodynamic correlations and assessing long-term safety will be critical to positioning these lozenges as viable therapeutic alternatives for managing oral and throat infections. previous study demonstrates the antimicrobial efficacy of polyherbal lozenges containing Ashwagandha, Neem, and Tulsi tinctures, with the B4 batch showing the highest bacterial inhibition (17.1 ± 0.07 mm). Compared to previous studies, individual extracts of these herbs have shown similar antimicrobial effects, with Neem and Tulsi exhibiting strong activity against E. coli and other pathogens. Polyherbal formulations with Neem and Tulsi have reported comparable inhibition zones (14-18 mm) and disintegration times (~4 min), aligning with our findings. Our study contributes to existing literature by introducing a novel combination in lozenge form, adhering to Indian Pharmacopeia and ICH guidelines, and providing a potential natural alternative to synthetic antimicrobial lozenges.[66] Both studies focus on developing antimicrobial herbal lozenges, but our study offers a more comprehensive evaluation. While the other study formulates tablet lozenges using Cinnamomum tamala and Spilanthes acmella via direct compression, our study develops chewable lozenges with Cardamom, Ginger, and Karpooravalli using the molding method. Both exhibit antibacterial activity against Staphylococcus aureus and Escherichia coli, with our formulation demonstrating a higher inhibition zone (25 mm for E. coli). Additionally, our study integrates in silico docking, identifying Rosmarinic acid as a key inhibitor of bacterial enzymes, alongside ADME profiling for pharmacokinetic assessment, whereas the other study focuses on phytochemical screening and physical properties. Our lozenges also achieve a rapid drug release (91.26% within 25 minutes), ensuring enhanced bioavailability. These findings highlight our formulation's potential as a scientifically validated natural therapy with superior antimicrobial efficacy.[67] Both studies focus on polyherbal lozenge formulations but differ in scope and evaluation. Our study targets mouth and throat infections using cardamom, ginger, and karpooravalli, demonstrating potent antimicrobial efficacy (S. aureus, E. coli) with 91.26% drug release in 25 minutes. In silico docking and ADME profiling identified rosmarinic acid as a key bioactive compound. In contrast, the compared study addresses cold and flu symptoms, formulating lozenges with herbal juices, jaggery, and sugar. While both assess quality parameters, our study integrates computational analysis and mechanistic insights, offering a more robust scientific foundation. [68,69]

Docking results, which predict the binding affinity and interaction patterns between ligands and target proteins, often correlate with experimental antimicrobial activity but are not definitive on their own. Compounds with strong binding energies, such as Rosmarinic Acid (-8.7 kcal/mol against MurA) and Gingerol (-7.0 kcal/mol), frequently demonstrate significant antibacterial activity in vitro, aligning with their predicted potency. However, some phytochemicals like β -Sitosterol (-7.2 kcal/mol against DHFR), despite lacking hydrogen bonds, still exhibit antimicrobial effects due to non-polar interactions or alternative mechanisms such as membrane disruption. Standard antibiotics like Fosfomycin (-7.5 to -8.5 kcal/mol) and Trimethoprim (-8.0 to -9.0 kcal/mol) serve as benchmarks, with many phytochemicals

showing comparable binding energies and experimental efficacy.[70] Nevertheless, factors like bioavailability, bacterial resistance, and pharmacokinetics can influence experimental outcomes, leading to discrepancies between docking predictions and actual activity. For example, poor solubility or rapid metabolism may limit efficacy despite strong in silico binding, while compounds with moderate binding energies may still be effective through synergistic or multi-target effects. Experimental validation through assays like MIC, time-kill studies, and in vivo models is essential to confirm the antimicrobial potential of these compounds[71]. Thus, while docking studies provide valuable insights, they must be complemented by experimental data to fully assess a compound's therapeutic potential.

Conclusion

This comprehensive study rigorously evaluated the quality, efficacy, and therapeutic potential of polyherbal chewable lozenges formulated for the treatment of mouth and throat infections. A series of critical quality control assessments, including weight variation, friability, moisture content, dissolution, antimicrobial activity, and in silico analyses, demonstrated that the lozenges comply with pharmaceutical standards while exhibiting significant therapeutic promise. The weight variation test confirmed dosage consistency, ensuring uniform therapeutic delivery, while friability and moisture content analyses supported the robustness and stability of the formulation. The dissolution profile indicated efficient release of active ingredients, with a cumulative release of 91.26% by 25 minutes, confirming the lozenges' potential for rapid therapeutic action. The antimicrobial assessment underscored the efficacy of the formulation, particularly against Escherichia coli and Staphylococcus aureus, with the latter exhibiting notable susceptibility. Molecular docking studies provided insights into the binding affinities of key phytochemicals, with rosmarinic acid (Karpuravalli) and betasitosterol (Cardamom) demonstrating strong inhibitory potential against bacterial enzymes. Furthermore, pharmacokinetic and drug-likeness evaluations highlighted the favorable properties of compounds such as carvacrol and gingerol, while bioactivity scores indicated diverse interaction potentials, contributing to the lozenges' overall antimicrobial efficacy. This study integrates traditional herbal knowledge with advanced computational techniques, offering a robust framework for the development of effective and safe therapeutic solutions. The findings advocate for further in vitro and in vivo investigations to validate therapeutic efficacy and optimize the formulation for clinical application. The successful incorporation of multiple phytochemicals into a single dosage form underscores the potential of these lozenges as a valuable addition to current treatments for oral infections. Moreover, this research supports the advancement of polyherbal formulations in modern therapeutics, reinforcing their role in addressing oral health challenges through an evidence-based approach. Further research is needed to confirm the effectiveness of this approach, as current findings are limited

Discourse Data

- Ethics approval and consent to participate: Not applicable
- Availability of data and materials: The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
- Author's contribution: The authors confirm contribution to the paper as follows: study conception and design: Zaid Khan, Ramya C V, theoretical calculations and modeling: Zaid Khan, Ramya C V; data analysis and validation, Zaid Khan, draft manuscript preparation: Zaid Khan, Ramya C V,

Deepthi Swapna P R, Viahwanath B A, All authors reviewed the results and approved the final version of the manuscript.

- Funding: Not applicable
- Conflicts of interest: The authors declare that there is no conflict of interest regarding the publication of this article
- Acknowledgements: Not applicable

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References

- Caruso, T. J., Prober, C. G., & Gwaltney, J. M. (2007). Treatment of Naturally Acquired Common Colds with Zinc: A Structured Review. Clinical Infectious Diseases/Clinical Infectious Diseases (Online. University of Chicago. Press), 45(5), 569–574. https://doi.org/10.1086/520031
- 2] Kurkin, V. A., Varina, N. R., Avdeeva, E. V., Klimova, L. D., Pervushkin, S. V., & Ryazanova, T. K. (2016). The Development of The Combined Medicinal Herbal Remedies for Stomatological and Otorhinolaryngological Practice. Nauka I Innovacii V Medicine, 1(4), 51–57. https://doi.org/10.35693/2500-1388-2016-0-4-51-57
- 3] Selvadurai, S., Divya, S., Geetha, A., Rohini, K., & Anbazhagan, S. (2020). Herbal excipients- Significance of substances of natural origin as excipients. International Journal of Pharmtech Research, 13(3), 242–252. https://doi.org/10.20902/ijptr.2019.130315
- 4] Al-Faris, E. A., Al-Rowais, N., Mohamed, A. G., Al-Rukban, M. O., Al-Kurdi, A., Al-Noor, M. a. B., Al-Harby, S., & Sheikh, A. (2008). Prevalence and pattern of alternative medicine use: the results of a household survey. Annals of Saudi Medicine/Annals of Saudi Medicine, 28(1), 4–10. https://doi.org/10.5144/0256-4947.2008.4
- 5] Haidopoulou, K., Goutaki, M., Damianidou, L., Eboriadou, M., Antoniadis, A., & Papa, A. (2010). Human bocavirus infections in hospitalized Greek children. Archives of Medical Science/Archives of Medical Science (Online), 1, 100–103. https://doi.org/10.5114/aoms.2010.13515
- 6] El-Sherif, A. M. (2022). Prevalence of tonsillitis among sore throat patients attending OPD Department at Zawia Central Hospital, Libya. Libyan Journal of Medical Research, 16(2), 166–176. https://doi.org/10.54361/ljmr.16216
- 7] Blicharz, M., Miazga, D., Bartosik-Zielińska, D., & Bartosik, M. (2023). Burning mouth syndrome - causes and modern treatment methods. Journal of Education, Health and Sport, 38(1), 218–229. https://doi.org/10.12775/jehs.2023.38.01.015
- 8] Thornton, H. V., Hay, A. D., Redmond, N. M., Turnbull, S. L., Christensen, H., Peters, T. J., Leeming, J. P., Lovering, A., Vipond, B., Muir, P., & Blair, P. S. (2017). Throat swabs in children with respiratory tract infection: associations with clinical presentation and potential targets for point-of-care testing. Family Practice, 34(4), 407– 415. https://doi.org/10.1093/fampra/cmw136
- 9] Zhang, Z., Zheng, Y., Jiang, L., Ji, H., Chen, G., Luo, P., Pan, J., Tian, X., Wei, L., Huo, D., Miao, Z., Zou, X., Chen, J., Liao, Q., & Chang, Z. (2019). [Review on the etiology and complications of hand, foot and mouth disease, using data from the national sentinel surveillance program, in China, 2015-2016]. PubMed, 40(6), 627– 632. https://doi.org/10.3760/cma.j.issn.0254-6450.2019.06.005
- 10] Bansal M, K Singh S, Gulati M. Lozenges as delivery system for upper respiratory catarrh medication. Recent Patents on Drug Delivery & Formulation. 2014 Aug 1;8(2):92-100. 10.2174/1872211308666140422150205
- 11] Kulkarni MP, Sharma A, Tanwar S, Vandana PB, Wadhwa S, Singh G, Kumar P, Kumar R. Pharmaceutical lozenges: recent trends and developments with an update on research and patents. Recent Advances in Drug Delivery and Formulation: Formerly Recent

Patents on Drug Delivery & Formulation. 2022 Mar 1;16(1):45-54. https://doi.org/10.2174/2667387816666211231103759

- 12] Bansal M, K Singh S, Gulati M. Lozenges as delivery system for upper respiratory catarrh medication. Recent Patents on Drug Delivery & Formulation. 2014 Aug 1;8(2):92-100. https://doi.org/10.2174/1872211308666140422150205
- 13] Timko BP, Kohane DS. Drug-Delivery Systems for Tunable and Localized Drug Release. Israel Journal of Chemistry. 2013 Oct;53(9-10):728-36. https://doi.org/10.1002/ijch.201300093
- 14] Bao L, Zhang C, Dong J, Zhao L, Li Y, Sun J. Oral microbiome and SARS-CoV-2: beware of lung co-infection. Frontiers in microbiology. 2020 Jul 31;11:1840. https://doi.org/10.3389/fmicb.2020.01840
- 15] Scapoli, L., Palmieri, A., Pellati, A., Carinci, F., Lauritano, D., Arcuri, C., Baggi, L., Gatto, R., & Martinelli, M. (2022). Prevalence of Staphylococcus aureus and mec-A Cassette in the Throat of Non-Hospitalized Individuals Randomly Selected in Central Italy. Antibiotics, 11(7), 949. https://doi.org/10.3390/antibiotics11070949
- 16] Padhiary, S. K., Srivastava, G., Panda, S., Subudhi, S., & Lenka, S. (2013). E.coli associated extensive bilateral maxillary osteomyelitis: a rare case report. Journal of Clinical and Diagnostic Research. https://doi.org/10.7860/jcdr/2013/5628.3531
- 17] Niculescu, A., & Grumezescu, A. M. (2021). Natural compounds for preventing Ear, Nose, and Throat-Related oral infections. Plants, 10(9), 1847. https://doi.org/10.3390/plants10091847
- 18] Mustafa Z, Ghaffari M. Diagnostic methods, clinical guidelines, and antibiotic treatment for group A streptococcal pharyngitis: a narrative review. Frontiers in Cellular and Infection Microbiology. 2020 Oct 15;10:563627. https://doi.org/10.3389/fcimb.2020.563627
- 19] Al-Manei K, Ghorbani M, Naud S, Al-Manei KK, Sobkowiak MJ, Lund B, Hazirolan G, Sällberg Chen M, Özenci V. Clinical microbial identification of severe oral infections by MALDI-TOF mass spectrometry in Stockholm County: an 11-Year (2010 to 2020) epidemiological investigation. Microbiology Spectrum. 2022 Dec 21;10(6):e02487-22. https://doi.org/10.1128/spectrum.02487-22
- 0] Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral microbiome: a review of its impact on oral and systemic health. Microorganisms. 2024 Sep;12(9):1797. https://doi.org/10.3390/microorganisms12091797
- Astuti, P., Sudarsono, S., Nisak, K., & Nugroho, G. W. (2014).
 Endophytic Fungi Isolated from Coleus amboinicus Lour Exhibited Antimicrobial Activity. DOAJ (DOAJ: Directory of Open Access Journals), 4(Suppl 2), 599–605.
 https://doi.org/10.5681/apb.2014.088
- 22] Shamna, K. P., & Poyil, M. M. (2021). Isolation of multidrug resistant bacterial pathogens and screening against Coleus amboinicus L. extracts. Annals of Phytomedicine-an International Journal, 409-415.http://dx.doi.org/10.21276/ap.2021.10.2.54
- 23] Rahmani AH, Aly SM. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International journal of physiology, pathophysiology and pharmacology. 2014 Jul 12;6(2):125.
- 24] Abdullah, N., Asghar, A., Butt, M. S., Shahid, M., & Huang, Q. (2017). Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of Chromobacterium violaceum. Journal of Food Science and Technology/Journal of Food Science and Technology, 54(8), 2306–2315. https://doi.org/10.1007/s13197-017-2668-7
- 25] Thurapati, P. R., Bongoni, R. N., & Mohan, R. (2019). Evaluation of antibacterial Activity of Cardamom Seed Powder Extract. ResearchGate. https://doi.org/10.20959/wjpr20198-15334
- 26] Masoumi-Ardakani, Y., Mahmoudvand, H., Mirzaei, A., Esmaeilpour, K., Ghazvini, H., Khalifeh, S., & Sepehri, G. (2017). The effect of Elettaria cardamomum extract on anxiety-like behavior in a rat model of post-traumatic stress disorder. Biomedicine & Pharmacotherapy, 87, 489–495. https://doi.org/10.1016/j.biopha.2016.12.116
- 27] Khatri, P., Rani, A., Hameed, S., Chandra, S., Chang, C., & Pandey, R. P. (2023). Current understanding of the molecular basis of spices for the development of potential antimicrobial medicine. Antibiotics, 12(2), 270. https://doi.org/10.3390/antibiotics12020270
- 28] Nuñez, L., & Aquino, M. D. (2012). Microbicide activity of clove essential oil (Eugenia caryophyllata). Brazilian Journal of Microbiology, 43(4), 1255–1260. https://doi.org/10.1590/s1517-83822012000400003
- 29] Zafar, F., Shahid, M., Fatima, H., Riaz, M., Anjum, F., Mushtaq, Z., Zia, S., Jahangir, M. M., & Aslam, M. A. (2022). Antibiofilm and Quorum Sensing Inhibition (QSI) Potential of Lagerstroemia speciosa Leaves Extract. Dose-response, 20(4), 155932582211320. https://doi.org/10.1177/15593258221132080
- Nzeako, B. C., Al-Kharousi, Z. S. N., & Al-Mahrooqui, Z. (2006, June 1). Antimicrobial activities of clove and thyme extracts. PubMed

Central

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3074903/

- 31] Arshad, S., Rehman, T., Saif, S., Rajoka, M. S. R., Ranjha, M. M. a. N., Hassoun, A., Cropotova, J., Trif, M., Younas, A., & Aadil, R. M. (2022). Replacement of refined sugar by natural sweeteners: focus on potential health benefits. Heliyon, 8(9), e10711. https://doi.org/10.1016/j.heliyon.2022.e10711
- 32] Monica K, Rajeshkumar S, Ramasubramanian A, Ramani P, Sukumaran G. Anti-inflammatory and antimicrobial effects of herbal formulation using karpooravalli, mint, and cinnamon on wound pathogens. Journal of advanced pharmaceutical technology & 2022 research Dec 1;13(Suppl 2):S369-73. 10.4103/japtr.japtr_515_22
- 33] Ballester P, Cerdá B, Arcusa R, García-Muñoz AM, Marhuenda J, Zafrilla P. Antioxidant activity in extracts from Zingiberaceae family: Cardamom, turmeric, and ginger. Molecules. 2023 11;28(10):4024. https://doi.org/10.3390/molecules28104024
- 34] Wadikar DD, Patki PE. Coleus aromaticus: a therapeutic herb with multiple potentials. Journal of food science and technology. 2016 Jul;53:2895-901. https://doi.org/10.1007/s13197-016-2292-y
- 35] Arshad, S., Rehman, T., Saif, S., Rajoka, M. S. R., Ranjha, M. M. a. N., Hassoun, A., Cropotova, J., Trif, M., Younas, A., & Aadil, R. M. (2022). Replacement of refined sugar by natural sweeteners: focus on potential health benefits. Heliyon, 8(9), e10711. https://doi.org/10.1016/j.heliyon.2022.e10711
- 36] Farag, M. A., Rezk, M. M., Elashal, M. H., El-Araby, M., Khalifa, S. a. M., & El-Seedi, H. R. (2022). An updated multifaceted overview of sweet proteins and dipeptides as sugar substitutes; the chemistry, health benefits, gut interactions, and safety. Food Research International, 162, 111853. https://doi.org/10.1016/j.foodres.2022.111853
- 37] N, D. G., Kajal, M., & S, K. S. (2021). REVIEW ON HERBAL LOZENGES PREPARED FOR COLD AND FLU. Journal of and Pharmaceutical Research, Biomedical https://doi.org/10.32553/jbpr.v10i3.869
- 38] Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. Methods in Molecular Biology, 365-382. https://doi.org/10.1007/978-1-59745-177-2 19
- 39] Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwuja, E. I., & Aja, P. M. (2023). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-40160-2
- 40] Meng, X., Zhang, H., Mezei, M., & Cui, M. (2011). Molecular Docking: a powerful approach for Structure-Based drug discovery. Current Computer - Aided Drug Design, 7(2), 146–157. https://doi.org/10.2174/157340911795677602
- 41] Tao, Y., Qu, H., Wang, S., Yan, F., Wang, C., & Zhang, M. (2022). Computational assessment of herbal medicine-derived compounds as potential inhibitors of SARS-CoV-2 main protease. Journal of Biomolecular Structure and Dynamics, 1 - 12https://doi.org/10.1080/07391102.2022.2144949
- 42] Kailey, R., Rasane, P., Singh, J., Kaur, S., Gunjal, M., Kaur, J. Bhadariya, V., & Avinashe, H. (2024). Herbal candies: a potential source of health benefits. Current Nutrition & Food Science, 20(9), 1039-1048
 - https://doi.org/10.2174/0115734013250026231016140631
- 43] Formulation of Ethanol Extract (Myrmecodia pendans) as an Antibacterial Streptococcus mutans in Chewable Lozenges for Children with Early Childhood Caries. (2020). Systematic Reviews in Pharmacy, 11(04). https://doi.org/10.31838/srp.2020.4.37
- 44] Prasad, N. K. J., Vulava, N. S., Konda, N. V. C. R., & Subash, N. K. (2022). Toxicological Analysis by Computational Assessment Tools of Herbal Medicine Compounds Urushiol II and Bhilawanol-B from Semecarpus Anacardium, Indian Journal of Forensic Medicine & Toxicology, 16(3), 17–21. https://doi.org/10.37506/ijfmt.v16i3.18241
- 45] Dabo, N. D. A., Zechariah, N. K. I., Pauline, N. G., Naomi, N. M. N., Dawal, N. S. P., & Vwamkat, N. a. S. (2023). Assessment of Microbial Contamination of Solid Herbal Medicine sold in Makurdi Metropolis. World Journal of Advanced Research and Reviews, 21(2), 496-505. https://doi.org/10.30574/wjarr.2024.21.2.0433
- 46] Wang Y, Wang X, Cheng Y. A computational approach to botanical drug design by modeling quantitative composition-activity relationship. Chemical biology & drug design. 2006 Sep;68(3):166-72. https://doi.org/10.1111/j.1747-0285.2006.00431.x
- 47] Singh H, Bharadvaja N. Treasuring the computational approach in medicinal plant research. Progress in Biophysics and Molecular Biology. 2021 Sep 1;164:19-32. 10.1016/j.pbiomolbio.2021.05.004
- 48] Kumbhar P, Pandey NK, Kumar B, Vinchurkar K. Artificial Intelligence in Herbal Medicine Formulations. InAl Innovations in Drug Delivery and Pharmaceutical Sciences; Advancing Therapy through

Technology 2024 Nov 18 (pp. 147-162). Bentham Science Publishers. 10.2174/9789815305753124010010

- 49] Xu C, Wu S, Liu P, Huang Y, Chen Y, Ding G, Jia S. Computational identification and analysis of CNP0269688 as a natural product inhibitor disrupting the interaction between the HIV matrix domain and tRNA. Frontiers in Chemistry. 2024 Sep 2;12:1450339. https://doi.org/10.3389/fchem.2024.1450339
- 50] Ningthoujam SS, Nath R, Sarker SD, Nahar L, Nath D, Talukdar AD. Prediction of medicinal properties using mathematical models and computation, and selection of plant materials. InComputational phytochemistry 2024 Jan 1 (pp. 91-123). Elsevier. 10.1016/B978-0-12-812364-5.00002-X
- 51] Romano JD, Tatonetti NP. Informatics and computational methods in natural product drug discovery: a review and perspectives. Frontiers genetics. 2019 30:10:368. Apr https://doi.org/10.3389/fgene.2019.00368
- 52] Medina-Franco JL. Computational approaches for the discovery and development of pharmacologically active natural products. Biomolecules. 2021 Apr 23,11(5):630. 10.3390/biom11050630
- Wang T, Wu Z, Sun L, Li W, Liu G, Tang Y. A computational systems 531 pharmacology approach to investigate molecular mechanisms of herbal formula Tian-Ma-Gou-Teng-Yin for treatment of Alzheimer's disease. Frontiers in pharmacology, 2018 Jun 26;9:668. https://doi.org/10.3389/fphar.2018.00668
- 54] Rehman, H., Zahoor, A., Shaikh, Z. A., Naveed, S., & Usmanghani, K. (2017). Polyherbal extract based Linkus lozenges for Symptomatic Relief: Design, development and evaluation. American Journal of Advanced Drug Delivery, 05(01). https://doi.org/10.21767/2321-547x.1000008
- 55] Bhat, J. G., Bhat, M. G., Bhat, S. K., Bhat, S., & Pujari, G. R. (2023). ANTICANCER PROPERTY OF AN AYURVEDIC POLYHERBAL PREPARATION CHIEFLY CONTAINING ARECA NUT AND BETEL LEAF. International Journal of Research in Ayurveda and Pharmacy, 14(1), 13-16. https://doi.org/10.7897/2277-4343.14015
- KIRAN RS, SHEKAR BC, BABU BN. International journal of 56] pharmaceutical sciences. Int. J. Ph. Sci. 2011 Jan;3(1)
- 57 Srivallika T, Trivedi MH, Srinadh M, Chinnaeswaraiah M. Formulation and evaluation of polyherbal lozenges
- Kumar A, Mishra MK, Afeefa CK, Pai G, Pai V. Development and 58] evaluation of polyherbal lozenges for cold and flu. Indian Journal of Pharmaceutical Education and Research. 2019 Apr 1;53(2):159-63.
 - Smith SM, Schroeder K, Fahey T. Over-the-counter (OTC) medications for acute cough in children and adults in community tings. Cochrane Database of Systematic Reviews. 2014(11). 10.1002/14651858.CD001831.pub5
- 60] Suga T, Okita K, Morita N, Yokota T, Hirabayashi K, Horiuchi M, Takada S, Omokawa M, Kinugawa S, Tsutsui H. Dose effect on intramuscular metabolic stress during low-intensity resistance exercise with blood flow restriction. Journal of applied physiology. 2010 Jun;108(6):1563-7. 10.1152/japplphysiol.00504.2009
- 61] Perez-Castro R, Patel S, Garavito-Aguilar ZV, Rosenberg A, Recio-Pinto E, Zhang J, Blanck TJ, Xu F. Cytotoxicity of local anesthetics in human neuronal cells. Anesthesia & Analgesia. 2009 Mar 1;108(3):997-1007. 10.1213/ane.0b013e31819385e1
- 62] Smith SM, Schroeder K, Fahey T. Over-the-counter (OTC) medications for acute cough in children and adults in community Cochrane Database Systematic settings. of Reviews. 2014(11). 10.1002/14651858.CD001831.pub5
- 63] Binder D, Hromatka O, Geissler F, Schmied K, Noe CR, Burri K, Pfister R, Strub K, Zeller P. Analogs and derivatives of tenoxicam. 1. Synthesis and antiinflammatory activities of analogs with different residues on the ring nitrogen and the amide nitrogen. Journal of chemistry. 1987 Apr;30(4):678-82. medicinal https://doi.org/10.1021/jm00387a017
- 64] Kim DH, Lees WJ, Kempsell KE, Lane WS, Duncan K, Walsh CT. Characterization of a Cys115 to Asp substitution in the Escherichia coli cell wall biosynthetic enzyme UDP-GIcNAc enolpyruvyl transferase (MurA) that confers resistance to inactivation by the antibiotic fosfomycin. Biochemistry. 1996 Apr 16;35(15):4923-8. https://doi.org/10.1021/bi952937w
- 65] Kim DH, Lees WJ, Kempsell KE, Lane WS, Duncan K, Walsh CT. Characterization of a Cys115 to Asp substitution in the Escherichia coli cell wall biosynthetic enzyme UDP-GlcNAc enolpyruvyl transferase (MurA) that confers resistance to inactivation by the antibiotic fosfomycin. Biochemistry. 1996 Apr 16;35(15):4923-8. https://doi.org/10.1021/bi952937w
- 66] Dhoke PS, Biyani DM, Maske AO, Umekar M, Hatwar B. Recent Formulation Development and Evaluation of Lozenges Containing

Polyherbal Extract of Cinnamomum tamala and Spilanthes acmella. https://doi.org/10.9734/bpi/caprd/v1/3953F

- 67] Solomon DJ, Björk BC. A study of open access journals using article processing charges. Journal of the American Society for Information Science and Technology. 2012 Aug;63(8):1485-95.
- 68] Dhoke PS, Biyani DM, Maske AO, Umekar M, Hatwar B. Recent Formulation Development and Evaluation of Lozenges Containing Polyherbal Extract of Cinnamomum tamala and Spilanthes acmella. https://doi.org/10.9734/bpi/caprd/v1/3953F
- 69] Kumar A, Mishra MK, Afeefa CK, Pai G, Pai V. Development and evaluation of polyherbal lozenges for cold and flu. Indian Journal of Pharmaceutical Education and Research. 2019 Apr 1;53(2):159-63.5530/ijper.53.2s.61
- 70] Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ. Microtubuleinterfering agents stimulate the transcription of cyclooxygenase-2: evidence for involvement of ERK1/2 AND p38 mitogen-activated protein kinase pathways. Journal of Biological Chemistry. 2000 May 19;275(20):14838-45. 10.1074/jbc.275.20.14838
- 71] Radulović NS, Miltojević AB, McDermott M, Waldren S, Parnell JA, Pinheiro MM, Fernandes PD, de Sousa Menezes F. Identification of a new antinociceptive alkaloid isopropyl N-methylanthranilate from the essential oil of Choisya ternata Kunth. Journal of ethnopharmacology. 2011 Jun 1;135(3):610-9. https://doi.org/10.1016/j.jep.2011.03.035