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Phytochemical profiling, antimicrobial activity and molecular docking of *Dhanwantharam Gulika* and *Krimisodhini Gulika*

Mohamed Ahadu Shareef ^{1,*}, Mohamed Zubair ², Irfan Navabshan ³
 and Mohamed Divan Masood ⁴

¹ Postgraduate and Research Department of Chemistry, The New College (Autonomous), Affiliated to the University of Madras, Chennai, 600 014, Tamil Nadu, India

² Corrosion Science and Technology Division, Materials Characterization Group, Indira Gandhi Centre for Atomic Research, Kalpakkam, 603 102, Tamil Nadu, India

³ Crescent School of Pharmacy, Buhari Syed Abdur Rahman Crescent Institute of Science and Technology, Chennai, 600 048, Tamil Nadu, India

⁴ School of Computer Applications, Buhari Syed Abdur Rahman Crescent Institute of Science and Technology, Chennai, 600 048, Tamil Nadu, India

* Corresponding author at: Postgraduate and Research Department of Chemistry, The New College (Autonomous), Affiliated to the University of Madras, Chennai, 600 014, Tamil Nadu, India.

e-mail: jasshaali@gmail.com (M.A. Shareef).

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ABSTRACT

GC-MS analysis revealed three chromatographic peaks in *Dhanwantharam Gulika* and eighteen in *Krimisodhini Gulika*. These yielded three and eighteen best-supported tentative assignments, respectively, together with up to nine and fifty-four total library candidates when alternative spectral matches were included. *Dhanwantharam Gulika* was predominantly characterised by monoterpenoid/borneol derivatives, which accounted for approximately 93.17% of the total peak area, whereas *Krimisodhini Gulika* exhibited a diverse phytochemical profile comprising terpenoids, long-chain fatty acids, phenyl propanoids, lactones, esters, and nitrogen-containing compounds, with *n*-hexadecanoic acid representing 38.18% of the peak area. Both formulations exhibited appreciable antimicrobial activity against the tested microorganisms. Molecular docking against gastric lipase produced a best Glide XP docking score of -4.074 kcal/mol, while the most favourable MM-GBSA binding free energy reached -31.57 kcal/mol among the screened constituents. These computational results indicate moderate predicted ligand-protein interactions and should be considered preliminary, requiring experimental validation.

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1. Introduction

The growing global burden of microbial infections and the emergence of antimicrobial resistance have intensified the search for alternative therapeutic agents derived from natural sources. In this context, traditional medicinal systems such as Ayurveda offer a rich repository of polyherbal formulations with longstanding traditional use [1]. The major ingredients of *Dhanwantharam Gulika* include Jeeraka (*Cuminum cyminum*), Ela (*Elettaria cardamomum*), Ajamoda (*Apium graveolens*), and Haritaki (*Terminalia chebula*). *Krimisodhini Gulika* is formulated with *Ocimum americanum*, *Ferula assa-foetida*, *Nigella sativa*, *Garcinia morella*, *Cinnabar*, *Croton tiglium*, *Butea monosperma*, and *Schrebera swietenoides* [2]. These herbal and mineral ingredients are described in traditional Ayurvedic practice; however, their contributions to the biological properties of the respective formulations require experimental evaluation. Among these, *Dhanwantharam Gulika* and

Krimisodhini Gulika, classical Ayurvedic preparations used in traditional practice, have attracted scientific interest. Despite their extensive traditional usage, systematic scientific validation of their phytochemical composition, biological activity, and molecular mechanisms remains limited. Therefore, a comprehensive investigation that integrates modern analytical and computational approaches is useful for evaluating their chemical composition and biological properties in relation to their traditional use [3] [4].

Recent advancements in analytical techniques such as gas chromatography-mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), UV-visible spectroscopy, and nuclear magnetic resonance (NMR) have enabled detailed characterisation of complex herbal matrices [5]. These techniques facilitate the identification of bioactive phytoconstituents and provide insights into their functional groups and structural features. Furthermore, complementary interactions among phytochemicals have been discussed in polyherbal

research; however, such interactions require direct experimental testing [6]. In parallel, *in vitro* antimicrobial assays and *in silico* molecular docking studies are commonly used to evaluate formulation-level antimicrobial activity and to predict ligand–target interactions, respectively [7,8].

In this study, a multidisciplinary approach was employed to investigate the phytochemical profile, spectroscopic characteristics, antimicrobial potential, and molecular docking interactions of *Dhanwantharam Gulika* and *Krimisodhini Gulika*. GC–MS analysis was utilised to elucidate the chemical composition of the formulations, revealing variations in phytochemical diversity and functional attributes. Spectroscopic techniques (FTIR, UV–Vis, and ^1H NMR) were applied to confirm the presence of key functional groups and structural motifs. The antimicrobial activity was evaluated against selected bacterial and fungal pathogens using the agar disc diffusion method. In contrast, molecular docking analysis was performed to assess the binding affinities of the identified compounds toward gastric lipase, an exploratory computational target. Therefore, the objective of the present study was to systematically characterize the phytochemical composition and spectroscopic features of *Dhanwantharam Gulika* and *Krimisodhini Gulika* using GC–MS, FTIR, UV–Vis, and ^1H NMR analyses; evaluate their *in vitro* antimicrobial activity against selected bacterial and fungal pathogens; and investigate the potential interactions of their tentatively identified phytoconstituents with gastric lipase using molecular docking and MM-GBSA calculations. In addition, *in silico* ADMET analysis was performed to assess the predicted drug-likeness and pharmacokinetic properties of selected constituents.

2. Experimental

2.1. Materials and analytical methods

The Ayurvedic formulations *Dhanwantharam Gulika* and *Krimisodhini Gulika* were manufactured by Arya Vaidya Sala, Kottakkal, Kerala, India, and purchased from an authorized dealer in Chennai, Tamil Nadu, India. All chemicals and reagents employed were of analytical grade. FTIR spectra were recorded using KBr pellets on a Shimadzu FTIR-8400 spectrophotometer over the range 200–4000 cm^{-1} [9]. UV–Vis spectra were recorded using a Shimadzu UV–Vis 1600A spectrophotometer over the range 200–1500 nm [10]. ^1H NMR spectra were acquired using a JEOL JNM-ECZ400S/L1 spectrometer, with TMS as the internal standard [11]. GC–MS analysis was performed at IIT Madras, Tamil Nadu, using an Agilent 8890 gas chromatograph coupled to an Agilent 5977 mass-selective detector.

2.2. GC–MS data analysis and tentative compound identification

GC–MS data were processed using the Agilent MassHunter data analysis method associated with the GC–MS system. The chromatographic data were acquired using an Agilent 8890 gas chromatograph coupled to an Agilent 5977 mass selective detector with electron ionisation (EI) at 70 eV, with mass spectra acquired in scan mode over an m/z range of 50–600. Mass spectral identification was performed using the NIST17 mass spectral libraries available in the data-processing system, including the main library and associated NIST mass spectral databases [12]. The automated library search was configured to search TIC peaks and return up to 3 candidate hits for each chromatographic peak, with a minimum spectrum match score of 600. For each candidate, the library search output included the Score, Reverse Score, and Probability (%), and these parameters were considered for tentative compound assignment. The candidate with the highest library match score was regarded as the best-supported tentative assignment,

whereas lower-ranked hits were considered alternative tentative candidates. In addition to the library match parameters, the tentative assignments were evaluated by considering the consistency of the observed mass spectral fragment ions with the corresponding library spectra and the agreement of chromatographic retention times with available literature information where applicable. Because authentic reference standards were not analyzed, and retention indices were not experimentally determined, compound identities were not considered confirmed. Accordingly, all GC–MS-based identifications are reported as tentative assignments. Multiple library candidates associated with a single chromatographic peak should not be interpreted as evidence that all listed compounds were simultaneously present; rather, they represent alternative spectral matches for that peak [13].

2.3. Test microorganisms

Antimicrobial activity was evaluated by the agar disc diffusion method against selected bacterial and fungal strains, with the zone of inhibition used as the primary indicator of antimicrobial activity [14]. The formulations *Dhanwantharam Gulika* and *Krimisodhini Gulika* were tested against *Streptococcus pneumoniae*, *Proteus mirabilis*, *Lactobacillus acidophilus*, and *Candida albicans*. The microbial strains were obtained from the National Chemical Laboratory (NCL), Pune, India.

2.3.1. Antimicrobial assay (Agar disk diffusion method)

The antimicrobial activity of *Dhanwantharam Gulika* and *Krimisodhini Gulika* was evaluated by the agar disc diffusion method following the standard procedure described by Bauer *et al.* [15], with appropriate modifications. The formulations were tested against *Streptococcus pneumoniae*, *Proteus mirabilis*, and *Lactobacillus acidophilus* for antibacterial activity and *Candida albicans* for antifungal activity.

2.3.2. Preparation of Mueller–Hinton agar medium

Mueller–Hinton agar (MHA) was prepared by dissolving 34.0 g of dehydrated medium in 1000 mL of distilled water. The medium was heated with continuous mixing until completely dissolved and sterilized by autoclaving at 121 °C and 15 psi for 15 min. After sterilization, the medium was cooled to approximately 45–50 °C and aseptically poured into sterile Petri plates (approximately 20–25 mL per plate). The plates were allowed to solidify at room temperature before use.

2.3.3. Preparation of nutrient broth

Nutrient broth was prepared by dissolving peptone (5.0 g), beef extract (3.0 g), and sodium chloride (5.0 g) in 1000 mL of distilled water. The pH was adjusted to 7.3 ± 0.1 at 25 °C. The medium was sterilized by autoclaving at 121 °C and 15 psi for 15 min and subsequently cooled before inoculation.

2.3.4. Preparation of test extracts and solutions

The powdered Ayurvedic formulations, *Dhanwantharam Gulika* and *Krimisodhini Gulika*, were subjected to Soxhlet extraction using methanol as the extraction solvent [16]. The resulting methanolic extracts were concentrated to remove the solvent, and the obtained dried extracts were used for antimicrobial testing. Test solutions were prepared at appropriate concentrations, and different volumes were used to impregnate discs depending on the microbial strain and the corresponding standard antibiotic/antifungal control. For *Streptococcus pneumoniae*, *Proteus mirabilis*, and *Candida albicans*, sterile Whatman No. 1 filter paper discs of 6 mm

diameter were impregnated with 10, 20, and 30 μL of the respective test solutions. For *Lactobacillus acidophilus*, the discs were impregnated with 5, 10, and 15 μL of the respective test solutions. The impregnated discs were dried under aseptic conditions before use. Discs treated with the corresponding solvent alone were used as negative controls.

2.3.5. Preparation and standardization of microbial inoculum

Fresh microbial cultures were used to prepare the inoculum. A few well-isolated colonies from each bacterial culture were aseptically transferred into sterile tubes containing 4 mL of nutrient broth and incubated until adequate turbidity was obtained. The turbidity of each suspension was adjusted to that of a 0.5 McFarland standard to obtain a standardized microbial inoculum.

2.3.6. Agar disk diffusion assay

The standardized microbial inoculum was uniformly spread over the surface of solidified Mueller–Hinton agar plates using a sterile cotton swab to obtain a confluent lawn culture [17]. The inoculated plates were allowed to stand for approximately 5 minutes to facilitate absorption of excess surface moisture. Sterile 6-mm Whatman No. 1 filter paper discs impregnated with the respective test solutions were aseptically placed on the inoculated agar surface using sterile forceps. For *Streptococcus pneumoniae*, *Proteus mirabilis*, and *Candida albicans*, 10, 20, and 30 μL of the test solutions were applied to each disc, whereas 5, 10, and 15 μL were applied for *Lactobacillus acidophilus*. The selected application volumes corresponded to the respective experimental conditions used for comparison with the standard antibiotic or antifungal discs. The discs were gently pressed to ensure complete contact with the agar surface. *Ciprofloxacin* was used as the positive antibacterial control against *Streptococcus pneumoniae* and *Proteus mirabilis*, whereas doripenem was used as the positive antibacterial control against *Lactobacillus acidophilus*. *Fluconazole* was used as the positive antifungal control against *Candida albicans*. Discs containing the corresponding solvent alone were used as negative controls. The plates were incubated at 37 °C for 24 h. Following incubation, the diameters of the zones of inhibition surrounding each disc were measured in millimetres (mm). All experiments were performed in triplicate ($n = 3$). The results were expressed as mean $\pm$ SD. Statistical analyses beyond the calculation of descriptive statistics were not performed.

2.4. Molecular docking and in silico analysis

2.4.1. Protein preparation

The three-dimensional crystal structure of gastric lipase was retrieved from the Protein Data Bank (PDB ID: 1HLG) [18]. The structure was obtained from Homo sapiens (human), and chain A was selected for molecular docking. The crystal structure had an experimental resolution of 3.00 Å. Protein preparation was performed using the Protein Preparation Wizard implemented in the Schrödinger suite [19]. Bond orders were assigned and corrected using the Chemical Component Dictionary (CCD), followed by the addition of missing hydrogen atoms. All non-protein heteroatoms other than the polypeptide chain, including the N-linked glycosylation (NAG) residues resolved on the protein surface, were removed during preprocessing, as they were not part of the selected ligand-binding region. No native co-crystallized small-molecule ligand was present in PDB 1HLG, and therefore no ligand removal or ligand-based receptor-grid generation was performed. Crystallographic water molecules were removed according to

the protein preparation protocol. The protonation states of ionizable residues were assigned using PROPKA at pH 7.0 [20]. The prepared protein was subsequently subjected to restrained energy minimization using the OPLS4 force field [21] until the heavy-atom root-mean-square deviation (RMSD) converged to 0.30 Å, thereby relieving unfavourable steric contacts while preserving the experimentally determined protein geometry [19].

2.4.2. Ligand preparation

The best-supported tentatively assigned GC–MS candidate compounds from *Dhanwantharam Gulika* and *Krimisodhini Gulika* were selected as ligands for the molecular docking study. The two-dimensional structures of the selected compounds were obtained from PubChem and converted into three-dimensional structures. Ligand preparation was performed using the LigPrep module of the Schrödinger suite [22]. Epik was employed to generate relevant ionization and protonation states at pH 7.0 $\pm$ 2.0, while possible tautomeric and stereoisomeric forms were generated where applicable. The resulting ligand structures were subjected to energy minimization using the OPLS4 force field before molecular docking [21].

2.4.3. Receptor grid generation

Because no co-crystallized ligand was available for receptor-grid generation, the potential binding site of gastric lipase was identified using the SiteMap module of the Schrödinger suite [23]. SiteMap analysis was performed on the prepared protein structure to identify potential ligand-binding cavities [23]. The highest-ranked pocket, with a SiteMap Dscore of 0.990, was selected as the putative docking site. The predicted pocket was lined by Asp36, Glu35, Tyr129, Lys128, Glu125, Asp124, Ile161, Thr165, Ala286, Trp289, Met297, His298, Tyr299, Pro305, Tyr306, Tyr307, and Asn308.

The receptor grid was centered at X = 57.77 Å, Y = 94.82 Å, and Z = 77.98 Å, with an enclosing box defined to accommodate ligands with a maximum length of 13 Å. Standard van der Waals scaling parameters were applied during receptor-grid generation. The selected SiteMap pocket was used consistently for all subsequent docking calculations. As the crystal structure represents a closed conformation in which the catalytic serine is covered by the lid region, the docking results are interpreted as predicted interactions within the selected SiteMap-identified pocket rather than direct evidence of binding to the canonical catalytic site.

2.4.4. Molecular docking

The prepared ligands were docked into the SiteMap-predicted binding pocket of gastric lipase using the Glide module of the Schrödinger suite [24]. Glide extra-precision (XP) docking was employed to predict favourable ligand-binding poses and evaluate the interactions between the phyto-constituents and gastric lipase [24,25]. Default Glide XP parameters were used, with a ligand van der Waals radii scaling factor of 0.80 and a partial atomic charge cutoff of 0.15. Flexible ligand sampling was enabled, and the single best-scoring pose for each ligand was retained for further analysis. The resulting protein–ligand complexes were ranked according to their Glide docking scores, with more negative scores indicating more favourable predicted binding interactions under the applied computational model. The highest-ranked poses were subsequently analysed for hydrogen-bonding, hydrophobic, π – π , and other relevant non-covalent interactions with amino acid residues within the predicted binding pocket.

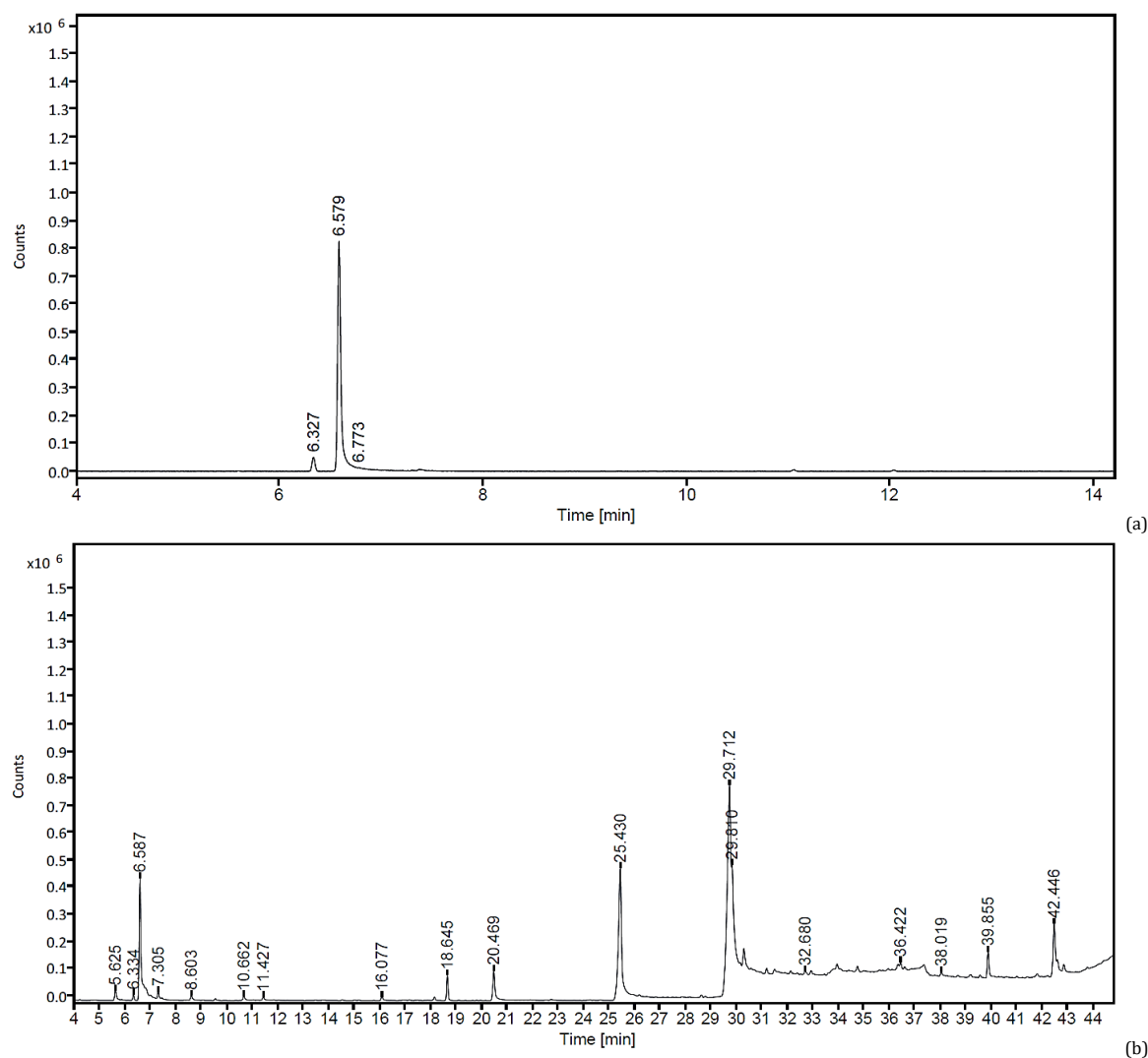


Figure 1. GC-MS chromatogram of (a) Ayurvedic *Dhanwantharam Gulika* and (b) Ayurvedic *Krimisodhini Gulika*.

2.4.5. Prime MM-GBSA binding free-energy calculation

The binding free energies of the selected protein-ligand complexes were further estimated using the Prime molecular mechanics/generalized Born surface area (MM-GBSA) method [26]. The optimized docked complexes were subjected to Prime MM-GBSA calculations to estimate the relative binding free energy (ΔG_{bind}) of each ligand-protein complex [26]. Calculations were performed using the OPLS4 force field [21] and the VSGB implicit solvation model [27]. The resulting ΔG_{bind} values were used to complement the Glide XP docking scores and to compare the predicted binding favourability of the selected phytoconstituents with gastric lipase. More negative ΔG_{bind} values were interpreted as indicating comparatively more favourable predicted binding under the applied computational model.

3. Results and discussion

3.1. GC-MS profiling of Ayurvedic formulations

Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed to elucidate the phytochemical composition of two Ayurvedic formulations, *Dhanwantharam Gulika* and *Krimisodhini Gulika*, obtained from Kottakkal Ayurvedic Medicine. The compositional and library-matching results are

presented in Tables 1–4: Tables 1 and 3 list the tentative compound assignments and associated chromatographic data, whereas Tables 2 and 4 provide the corresponding library-match parameters and alternative candidates. The GC-MS chromatographic profiles of *Dhanwantharam Gulika* and *Krimisodhini Gulika* are shown in Figures 1a and 1b, respectively. GC-MS analysis of *Dhanwantharam Gulika* and *Krimisodhini Gulika* revealed distinct phytochemical architectures, characterised by marked differences in chromatographic complexity and compound diversity. *Dhanwantharam Gulika* exhibited three chromatographic peaks with three best-supported tentative assignments and up to nine total library candidates, whereas *Krimisodhini Gulika* showed eighteen chromatographic peaks with eighteen best-supported tentative assignments and up to fifty-four total library candidates when alternative spectral matches were included. The identified constituents and their relative peak areas are summarized in Tables 1 and 3. Several compounds tentatively assigned by GC-MS, including camphor, isoborneol and borneol-related compounds, have previously been reported to possess antimicrobial activity. Their presence in the formulation may therefore provide a basis for hypothesizing a possible contribution to the observed antimicrobial activity. However, the present GC-MS analysis does not establish a causal relationship between any individual compound and the observed antimicrobial activity.

Table 1. GC-MS library-based tentative identification of compounds in *Dhanwantharam Gulika* by GC-MS analysis.

Peak Code	Ret. time (min)	Peak area (%)	Molecular weight (m/z)	Molecular formula	Compound name	Compound nature	PubChem CID
D1a	6.327	5.02	152.23	C ₁₀ H ₁₆ O	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)	Bicyclic monoterpenoid	444294
D1b			152.23	C ₁₀ H ₁₆ O	Camphor	Bicyclic monoterpene ketone	2537
D1c			152.23	C ₁₀ H ₁₆ O	2-Bornanone/Camphor	Bicyclic monoterpene ketone	2537
D2a	6.579	93.17	154.25	C ₁₀ H ₁₈ O	Isoborneol	Terpene	6321405
D2b			154.25	C ₁₀ H ₁₈ O	endo-Borneol	Bicyclic monoterpenoid	64685
D2c			154.25	C ₁₀ H ₁₈ O	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	Terpene	1201518
D3a	6.773	1.81	182.26	C ₁₁ H ₁₈ O ₂	Isobornyl formate	Monoterpenoid ester	23623868
D3b			172.69	C ₁₀ H ₁₇ Cl	Bornyl chloride	Bicyclic monoterpenoid	12300207
D3c			182.26	C ₁₁ H ₁₈ O ₂	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-formate, endo-	Borneol formate	518472

Table 2. GC-MS library matching results showing the best-supported tentative assignment and alternative candidate compounds for each chromatographic peak.

Peak code	RT (min)	Best-supported tentative assignment	Score	Reverse score	Probability (%)	Alternative library candidates for the same peak	Assignment status
D1a	6.327	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	941	954	47.21	Camphor (Score 929; Reverse Score 929; Probability 31.44%); 2-Bornanone/Camphor (Score 909; Reverse Score 909; Probability 14.32%)	Best-supported tentative library assignment; alternatives are candidate library matches for the same chromatographic peak and are not interpreted as simultaneously present compounds.
D2a	6.579	Isoborneol	911	913	27.96	endo-Borneol (Score 906; Reverse Score 906; Probability 22.54%); Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (Score 896; Reverse Score 899; Probability 15.91%)	Best-supported tentative library assignment; alternatives are candidate library matches for the same chromatographic peak and are not interpreted as simultaneously present compounds.
D3a	6.773	Isobornyl formate	795	795	14.70	Bornyl chloride (Score 787; Reverse Score 813; Probability 10.96%); Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo- (Score 784; Reverse Score 784; Probability 9.69%)	Best-supported tentative library assignment; alternatives are candidate library matches for the same chromatographic peak and are not interpreted as simultaneously present compounds.

3.2. GC-MS data analysis and tentative compound identification

The GC-MS data were analyzed using the data-analysis method associated with the Agilent GC-MS system. Mass spectral library searching was performed using the NIST17 MS Search libraries available in the analytical data-processing system [12]. The automated library search was configured to return up to 3 candidate hits for each chromatographic peak, with a minimum spectrum match score of 600. For each candidate, the library search output reported the Score, Reverse Score, and Probability (%), and these values were considered during tentative compound assignment. For each chromatographic peak, the highest-scoring library hit was considered the best-supported tentative assignment, whereas additional hits were treated as alternative candidates. A chromatographic peak was not interpreted as evidence that all library candidates were simultaneously present. Accordingly, all compounds identified by GC-MS library matching are described as tentative assignments, as their identities were not confirmed using authentic reference standards [28].

Each peak code represents a single chromatographic peak. The compound listed under "Best-supported tentative assignment" corresponds to the highest-scoring library match and should be regarded as a tentative identification rather than a confirmed compound identification. The alternative candidates are additional library matches for the same chromatographic peak and are reported to demonstrate the uncertainty of library-based identification; they should not be interpreted as evidence that all listed candidates are simultaneously present in the sample. Score, Reverse Score, and Probability (%) are reported as provided by the GC-MS library search [12]. The GC-MS chromatographic peaks observed for *Dhanwantharam Gulika* and *Krimisodhini Gulika* were subjected

to library matching against the NIST mass spectral library [12]. For each chromatographic peak, the highest-scoring library hit was designated as the best-supported tentative assignment, while other closely matching compounds were reported as alternative candidate assignments [13]. The corresponding library match parameters and candidate compounds are presented in Table 2 for *Dhanwantharam Gulika* and Table 4 for *Krimisodhini Gulika*. Because several structurally related compounds may produce similar mass spectra, the presence of multiple candidate compounds listed for a single chromatographic peak should not be interpreted as evidence that all candidates are simultaneously present. Therefore, all compound identifications reported in this study are considered tentative and based on library spectral matching; confirmation would require analysis using authentic reference standards and/or additional orthogonal analytical approaches.

The GC-MS analysis provided tentative library-based assignments of several compounds present in the tested formulation. However, the detection of these compounds does not establish that they are responsible for the observed antimicrobial activity, nor does it demonstrate synergistic or additive interactions among them. The biological activity observed in the present study should therefore be interpreted at the level of the tested formulation rather than attributed to individual tentatively identified compounds. Some of the identified compounds have been previously reported in the literature to possess antimicrobial or other relevant biological properties; therefore, their presence provides a basis for proposing possible contributions to the observed activity. Nevertheless, these interpretations remain literature-based hypotheses and require confirmation using purified compounds, concentration-response studies, and appropriate combination/synergy assays.

Table 3. GC-MS library-based tentative identification of compounds in *Krimisodhini Gulika* by GC-MS analysis.

Peak Code	Ret. time (min)	Peak area (%)	Molecular weight (m/z)	Molecular formula	Compound name	Compound nature	PubChem CID
K1a	5.625	1.57	126.11	C ₆ H ₆ O ₃	Maltol	Pyranones	8369
K1b			168.15	C ₈ H ₈ O ₄	3-Acetoxy-2-methyl-pyran-4-one	Pyranones	120048
K1c			126.11	C ₅ H ₆ N ₂ O ₂	Pyrimidine-4,6-diol, 5-methyl-	Pyrimidine	581007
K2a	6.334	0.71	152.23	C ₁₀ H ₁₆ O	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)	Bicyclic monoterpenoids	444294
K2b			152.23	C ₁₀ H ₁₆ O	Camphor	Cyclic monoterpene ketone	2537
K2c			152.23	C ₁₀ H ₁₆ O	(+)-2-Bornanone	Terpenoid ketone	159055
K3a	6.587	20.90	154.25	C ₁₀ H ₁₈ O	Isoborneol	Terpene derivative	6321405
K3b			154.25	C ₁₀ H ₁₈ O	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	Monoterpenoid	1201518
K3c			154.25	C ₁₀ H ₁₈ O	endo-Borneol	Bicyclic monoterpene	64685
K4a	7.305	0.81	154.25	C ₁₀ H ₁₈ O	α-Terpineol	Monoterpenols	17100
K4b			154.25	C ₁₀ H ₁₈ O	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, (R)-	Terpenoids	442501
K4c			154.25	C ₁₀ H ₁₈ O	L-α-Terpineol	Monoterpenols	443162
K5a	8.603	0.87	136.15	C ₈ H ₈ O ₂	Benzene acetic acid	Aromatic carboxylic acids.	999
K5b			159.19	C ₁₀ H ₉ NO	3-Oxo-4-phenylbutyronitrile	Nitriles	562666
K5c			180.16	C ₉ H ₈ O ₄	Propanedioic acid, phenyl-	Dicarboxylic acid	75791
K6a	10.662	0.81	150.18	C ₉ H ₁₀ O ₂	Hydrocinnamic acid	Phenylpropanoid	107
K6b			255.97	C ₉ H ₉ AgO ₂	Benzenepropanoic acid, silver(1+) salt	3-phenylpropionic acid	53169
K6c			255.27	C ₁₅ H ₁₃ NO ₃	4-Nitrosophenyl-β-phenylpropionate	Ester	562003
K7a	11.427	0.66	172.27	C ₁₀ H ₂₀ O ₂	n-Decanoic acid	Saturated fatty acid	2969
K7b			172.27	C ₁₀ H ₂₀ O ₂	8-Methylnonanoic acid	Fatty acid	111470
K7c			186.30	C ₁₁ H ₂₂ O ₂	Undecanoic acid	Saturated fatty acid	8180
K8a	16.077	0.69	200.32	C ₁₂ H ₂₄ O ₂	Dodecanoic acid	Saturated fatty acid	3893
K8b			214.35	C ₁₃ H ₂₆ O ₂	Tridecanoic acid	Saturated fatty acid	12530
K8c			242.40	C ₁₅ H ₃₀ O ₂	Pentadecanoic acid	Saturated fatty acid	13849
K9a	18.645	3.32	198.31	C ₁₂ H ₂₂ O ₂	γ-Dodecalactone	Lactone derivative	16821
K9b			226.36	C ₁₄ H ₂₆ O ₂	γ-Tetradecalactone	Cyclic ester / lactone / Fatty acid	97746
K9c			184.27	C ₁₁ H ₂₀ O ₂	2(3H)-Furanone, 5-heptyldihydro-	Butenolide	7714
K10a	20.469	5.91	228.38	C ₁₄ H ₂₈ O ₂	Tetradecanoic acid	Saturated fatty acid	11005
K10b			242.40	C ₁₅ H ₃₀ O ₂	Pentadecanoic acid	Saturated, long-chain fatty acid with 15 carbon atoms,	13849
K10c			214.35	C ₁₃ H ₂₆ O ₂	Tridecanoic acid	Saturated fatty acid	12530
K11a	25.430	38.18	256.43	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid	Saturated fatty acid	985
K11b			652.95	C ₃₈ H ₆₈ O ₈	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Derivative of vitamin C	168814
K11c			494.85	C ₃₂ H ₆₂ O ₃	Palmitic anhydride	Saturated fatty acid	69339
K12a	29.712	11.54	280.45	C ₁₈ H ₃₂ O ₂	9(E),11(E)-Conjugated linoleic acid	Unsaturated fatty acid	5282796
K12b			280.45	C ₁₈ H ₃₂ O ₂	10(E),12(Z)-Conjugated linoleic acid	Unsaturated fatty acid	5282800
K12c			280.45	C ₁₈ H ₃₂ O ₂	Linoelaidic acid	Unsaturated fatty acid	5282457
K13a	29.810	1.36	280.45	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid (Z,Z)-	Unsaturated fatty acid	5280450
K13b			280.45	C ₁₈ H ₃₂ O ₂	17-Octadecynoic acid	Fatty acid	1449
K13c			280.45	C ₁₈ H ₃₂ O ₂	(Z)-18-Octadec-9-enolide	Lactone derivative	6428982
K14a	32.680	0.69	334.54	C ₂₂ H ₃₈ O ₂	Cyclopropane octanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-methyl-ester	Cyclopropane fatty acid esters	534619
K14b			280.45	C ₁₈ H ₃₂ O ₂	Ethyl 9,12- hexadecadienoate	Polyunsaturated fatty acid methyl ester	141760622
K14c			310.52	C ₂₀ H ₃₈ O ₂	Ethanol, 2-(9,12- octadecadienyloxy)-, (Z,Z)	Alcohol	5365675
K15a	36.422	1.29	268.44	C ₁₇ H ₃₂ O ₂	7-Methyl-Z-tetradecen-1-ol acetate	Unsaturated fatty ester derivatives	5363222
K15b			280.50	C ₁₉ H ₃₆ O	12-Methyl-E,E-2,13- octadecadien-1-ol	Unsaturated fatty alcohol	90107969
K15c			268.44	C ₁₇ H ₃₂ O ₂	E-8-Methyl-9-tetradecen-1-ol acetate	Unsaturated fatty ester derivatives	5363273
K16a	38.019	0.70	268.44	C ₁₇ H ₃₂ O ₂	7-Methyl-Z-tetradecen-1-ol acetate	Unsaturated fatty ester derivatives	5363222
K16b			280.50	C ₁₉ H ₃₆ O	12-Methyl-E,E-2,13-octadecadien-1-ol	Fatty alcohol	90107969
K16c			338.53	C ₂₁ H ₃₈ O ₃	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	Unsaturated fatty acid ester derivatives	62574
K17a	39.855	4.37	392.67	C ₂₆ H ₄₈ O ₂	Borneol palmitate	Bicyclic alcohol	Not available
K17b			418.71	C ₂₈ H ₅₀ O ₂	Geranyl oleate	Ester	91694967
K17c			308.51	C ₂₀ H ₃₆ O ₂	Isobornyl caprate	Terpenoid isoborneol	91715501
K18a	42.446	5.62	278.41	C ₁₆ H ₂₂ O ₂ S	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	Aromatic sulfones	586248
K18b			236.39	C ₁₆ H ₂₈ O	3-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl-,	Terpene alcohol	Not available
K18c			235.17	C ₁₀ H ₁₉ BrO	5-(1-Bromo-1-methyl-ethyl)-2-methyl-cyclohexanol	Cyclic alcohol	586211

3.3. Phytochemical composition and functional interpretation

The phytochemical profile of *Dhanwantharam Gulika* was predominantly composed of monoterpenoids, with isoborneol and borneol derivatives constituting approximately 93.17% of the total peak area, followed by camphor (5%) and minor esters such as isobornyl formate. The predominance of these volatile terpenes shows a comparatively narrow tentative chemical profile. Monoterpenoids such as borneol have been investi-

gated for effects on membrane permeability and drug transport [29]; however, these literature reports do not establish such effects for the tested formulation. Additionally, these compounds have been investigated for antimicrobial, antiviral, and neuroprotective activities and for effects involving membrane function and oxidative-stress pathways [30]. The high relative abundance of borneol derivatives provides a basis for further compound-specific investigation, but their contribution to the formulation-level activity remains unconfirmed.

Table 4. GC–MS library matching results showing the best-supported tentative assignment and alternative candidates for each chromatographic peak.

Peak code	RT (min)	Best-supported tentative assignment	Score	Reverse score	Probability (%)	Alternative library candidates for the same peak	Assignment status
K1a	5.625	Maltol	877	900	83.74	3-Acetoxy-2-methyl-pyran-4-one (803; 822; 11.14%); Pyrimidine-4,6-diol, 5-methyl- (751; 770; 2.22%)	Best-supported tentative assignment
K2a	6.334	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	917	927	48.31	Camphor (898; 898; 23.46%); (+)-2-Bornanone (891; 891; 17.97%)	Best-supported tentative assignment
K3a	6.587	Isoborneol	935	936	44.06	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-; endo-Borneol	Best-supported tentative assignment
K4a	7.305	α -Terpineol	875	896	29.44	3-Cyclohexene-1-methanol, α,α ,4-trimethyl-, (R)-; L- α -Terpineol	Best-supported tentative assignment
K5a	8.603	Benzeneacetic acid	859	867	36.82	3-Oxo-4-phenylbutyronitrile (850; 881; 26.73%); Propanedioic acid, phenyl- (849; 857; 25.69%)	Best-supported tentative assignment
K6a	10.662	Hydrocinnamic acid	855	863	37.32	Benzenepropanoic acid, silver(1+) salt (854; 858; 35.87%); 4-Nitrosophenyl- β -phenylpropionate (822; 829; 9.58%)	Best-supported tentative assignment
K7a	11.427	n-Decanoic acid	848	848	79.26	8-Methylnonanoic acid; Undecanoic acid	Best-supported tentative assignment
K8a	16.077	Dodecanoic acid	818	842	83.43	Tridecanoic acid (705; 739; 5.13%); Pentadecanoic acid (691; 704; 3.21%)	Best-supported tentative assignment
K9a	18.645	γ -Dodecalactone	865	890	65.61	γ -Tetradecalactone (832; 859; 17.17%); 2(3H)-Furanone, 5-heptyldihydro- (806; 855; 5.14%)	Best-supported tentative assignment
K10a	20.469	Tetradecanoic acid	904	906	84.37	Pentadecanoic acid; Tridecanoic acid	Best-supported tentative assignment
K11a	25.430	n-Hexadecanoic acid	885	891	66.83	l-(+)-Ascorbic acid 2,6-dihexadecanoate; Palmitic anhydride	Best-supported tentative assignment
K12a	29.712	9(E),11(E)-Conjugated linoleic acid	899	912	22.67	10(E),12(Z)-Conjugated linoleic acid; Linoelaidic acid	Best-supported tentative assignment
K13a	29.810	9,12-Octadecadienoic acid (Z,Z)-	825	827	9.60	17-Octadecynoic acid (819; 842; 7.54%); (Z)-18-Octadec-9-enolide (813; 843; 5.93%)	Best-supported tentative assignment
K14a	32.680	Cyclopropanoic acid, 2-[[2-[(2-ethylcyclo propyl) methyl]cyclo propyl] methyl]-, methyl ester	751	804	11.12	Ethyl 9,12-hexadecadienoate (747; 801; 9.39%); Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- (738; 738; 6.82%)	Best-supported tentative assignment
K15a	36.422	7-Methyl-Z-tetradecen-1-ol acetate	756	808	27.69	12-Methyl-E,E-2,13-octadecadien-1-ol (750; 804; 21.76%); E-8-Methyl-9-tetradecen-1-ol acetate (701; 780; 4.47%)	Best-supported tentative assignment
K16a	38.019	7-Methyl-Z-tetradecen-1-ol acetate	695	766	23.29	12-Methyl-E,E-2,13-octadecadien-1-ol (692; 771; 20.58%); 9-Octadecenoic acid (Z)-, oxiranylmethyl ester (665; 695; 6.05%)	Best-supported tentative assignment
K17a/b	39.855	Borneol palmitate / Geranyl oleate	650 / 650	859 / 689	7.27 / 7.27	Isobornyl caprate (634; 814; 4.19%)	No clear single best-supported assignment; two top candidates have identical Score and Probability
K18a	42.446	(2,6,6-Trimethylcyclohex-1-enyl)methanesulfonyl benzene	677	828	12.49	3-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl- (666; 831; 8.56%); 5-(1-Bromo-1-methyl-ethyl)-2-methylcyclohexanol (656; 802; 6.05%)	Best-supported tentative assignment

In contrast, *Krimisodhini Gulika* demonstrated a chemically diverse profile comprising terpenoids, long-chain fatty acids, phenylpropanoids, lactones, esters, and nitrogen-containing compounds. Major constituents such as *n*-hexadecanoic acid (38.18%), isoborneol derivatives (20.90%), conjugated linoleic acids (11.54%), and tetradecanoic acid (5.91%) indicate the presence of both volatile and non-volatile bioactive compounds. Long-chain fatty acids have been reported to exhibit antimicrobial effects, including effects on microbial membranes and enzyme activity [31], while conjugated linoleic acid derivatives have been associated in previous studies with anti-inflammatory and anticancer-related effects involving lipid metabolism and cytokine signaling [32]. The coexistence of multiple chemical classes suggests that *Krimisodhini Gulika* is chemically diverse; however, the biological contributions of its constituents and their combined effects require direct experimental evaluation.

3.4. Comparative phytochemical analysis

Comparative analysis highlights a clear distinction between the two formulations in terms of chemical composition and functional orientation. *Dhanwantharam Gulika*, with its limited number of compounds and high monoterpenoid dominance, shows a comparatively narrow tentative chemical profile. In

contrast, *Krimisodhini Gulika* exhibits extensive phytochemical diversity, indicating greater tentative chemical diversity. Such differences underscore the importance of compositional complexity in influencing formulation-level activity; however, additive or synergistic effects were not evaluated in the present study. The dominance of fatty acids and terpenoids in *Krimisodhini Gulika* further provides hypotheses for further formulation-level and compound-specific investigation.

3.5. Biological context and mechanistic hypotheses

The GC–MS analysis provided tentative library-based assignments of several phytochemical constituents in *Dhanwantharam Gulika* and *Krimisodhini Gulika*. Some of the tentatively assigned compounds, including camphor, isoborneol, borneol-related compounds, terpenoids, fatty acids and lactones, have been reported in the literature [31,33] to possess antimicrobial or other biological activities [31]. These previous reports provide a basis for hypothesizing that some constituents of the formulations may contribute to the antimicrobial activity observed in the present study. However, the present GC–MS analysis does not establish a causal relationship between any individual tentatively identified compound and the observed antimicrobial activity. Similarly, the presence of multiple constituents does not by itself

demonstrate synergistic, additive or antagonistic interactions. Such interactions would require direct experimental evaluation using purified compounds, defined combinations and appropriate antimicrobial synergy assays. Therefore, the biological relevance of the GC-MS findings should be considered a literature-based hypothesis rather than evidence of individual compound activity or synergistic action within the formulations.

3.6. Retention time and physicochemical distribution

The retention-time distribution observed in the GC-MS analysis reflects the physicochemical diversity of the tentatively assigned constituents. Early-eluting peaks (approximately 5–7 min) were associated predominantly with lower-molecular-weight volatile compounds, including monoterpenoid-related constituents, whereas later-eluting peaks were associated mainly with less volatile compounds such as long-chain fatty acids and their derivatives [13]. This distribution illustrates the chemical diversity resolved by the GC-MS method; however, the individual compound assignments remain tentative because authentic reference standards and experimentally determined retention indices were not used for confirmation.

3.7. Interpretive limitations and future directions

The GC-MS profiles provide information on the chemical composition of *Dhanwantharam Gulika* and *Krimisodhini Gulika* and identify several constituents that have previously been investigated for biological activities. The antimicrobial experiments in the present study demonstrate activity of the formulations against the tested microorganisms; however, the contribution of individual tentatively assigned compounds to this activity cannot be established from the present data. Likewise, the GC-MS results do not provide evidence for rapid onset of action, neuroprotective effects, enhanced therapeutic efficacy, or multi-target activity in vivo. These possibilities may be considered hypotheses based on previously reported activities of related compounds [31,33], but they require direct experimental validation. Future studies should therefore include quantitative chemical characterization, isolation or purification of relevant constituents, compound-specific antimicrobial testing, combination/synergy assays, and appropriate in vivo investigations to determine their biological and pharmacological relevance.

3.8. Spectroscopic investigations

3.8.1. FTIR spectral analysis of *Dhanwantharam Gulika* and *Krimisodhini Gulika*

Fourier-transform infrared (FTIR) spectroscopy was employed to elucidate the functional groups present in the Ayurvedic formulations *Dhanwantharam Gulika* and *Krimisodhini Gulika* in the solid state. The FTIR spectrum of *Dhanwantharam Gulika* exhibited thirteen characteristic absorption bands at 3717 cm^{-1} (O–H stretching, sharp), 3520 cm^{-1} (O–H stretching, broad), 3384 cm^{-1} (N–H stretching), 3262 cm^{-1} (C–H stretching), 2919 cm^{-1} (aliphatic C–H), 2418 cm^{-1} (C–H), 2006 cm^{-1} (C=C=N), 1884 cm^{-1} (anhydride C=O), 1619 cm^{-1} (C=C stretching), 1104 cm^{-1} (=C–H bending), 1008 cm^{-1} (C–O stretching), and 584–576 cm^{-1} (C–X stretching) [9]. These bands are consistent with hydroxyl-, amine-, carbonyl-, and halogen-containing functional groups [9]. Similarly, the FTIR spectrum of *Krimisodhini Gulika* revealed twelve prominent peaks at 3871 cm^{-1} (O–H stretching), 3311 cm^{-1} (N–H stretching), 2924 cm^{-1} (–CH₂– stretching), 2475 cm^{-1} (S–H stretching), 2014 cm^{-1} (C=C=C), 1702 cm^{-1} (C=O stretching), 1628 cm^{-1} (C=C stretching), 1444 cm^{-1} (C–H bending), 1050 cm^{-1} (CO–O–CO stretching), 792 cm^{-1} (C=C bending), 711 cm^{-1}

(C–H), and 592 cm^{-1} (C–X stretching) [9]. These spectral features suggest the possible presence of alcohol-, amine-, thio-, carbonyl-, and aromatic-group environments [9].

3.8.2. Electronic absorption spectral analysis

The UV-Vis absorption spectra of both formulations were recorded in methanol at ambient temperature to investigate their electronic transitions. *Dhanwantharam Gulika* displayed absorption bands at 364 and 414 nm, which are attributed to $n \rightarrow \pi$ transitions, indicating the presence of non-bonding electrons associated with carbonyl or heteroatom-containing chromophores. In contrast, *Krimisodhini Gulika* exhibited absorption maxima at 239 nm ($\pi \rightarrow \pi$ transition) and 364 nm ($n \rightarrow \pi$ transition), suggesting the presence of conjugated systems and heteroatom-containing functional groups [10]. The observed spectral features are consistent with broad chromophoric and conjugated functionalities in both formulations [10]; however, UV-Vis data alone do not establish the identities of specific compound classes.

3.8.3. ¹H NMR spectral analysis

The proton nuclear magnetic resonance (¹H NMR) spectra of both formulations were recorded in DMSO-*d*₆ at 400 MHz. The ¹H NMR spectrum of *Dhanwantharam Gulika* showed signals at δ 3.34 ppm, 2.50 ppm, and a multiplet at δ 1.23–0.75 ppm. Because the formulation is a complex mixture and residual DMSO-*d*₆ occurs near δ 2.50 ppm, we treated these resonances as general spectral features rather than definitive –OH, –NH₂, or –CH₃ assignments [11,34]. The ¹H NMR spectrum of *Krimisodhini Gulika* exhibited signals at δ 5.33–5.31 ppm (t, olefinic protons), 3.33 ppm (s, –CH₂–), 2.50–1.90 ppm (m, –CH₂–), 1.49–1.23 ppm (t, –CH₂–), and 0.87–0.83 ppm (t, –CH₃). These signals are tentatively consistent with aliphatic-chain, unsaturated, and terminal-methyl proton environments [11]. The spectroscopic characterization further supported the presence of diverse functional groups and structural features in both Ayurvedic formulations. FTIR analysis revealed characteristic absorption bands corresponding to hydroxyl, carbonyl, amine, and aliphatic functional groups, while UV-Vis spectroscopy showed characteristic absorption maxima associated with the chromophoric constituents. The ¹H NMR spectra were consistent with broad aliphatic and olefinic proton environments [11] but did not confirm individual molecular structures.

3.9. Antimicrobial activity

3.9.1. In vitro antimicrobial assay

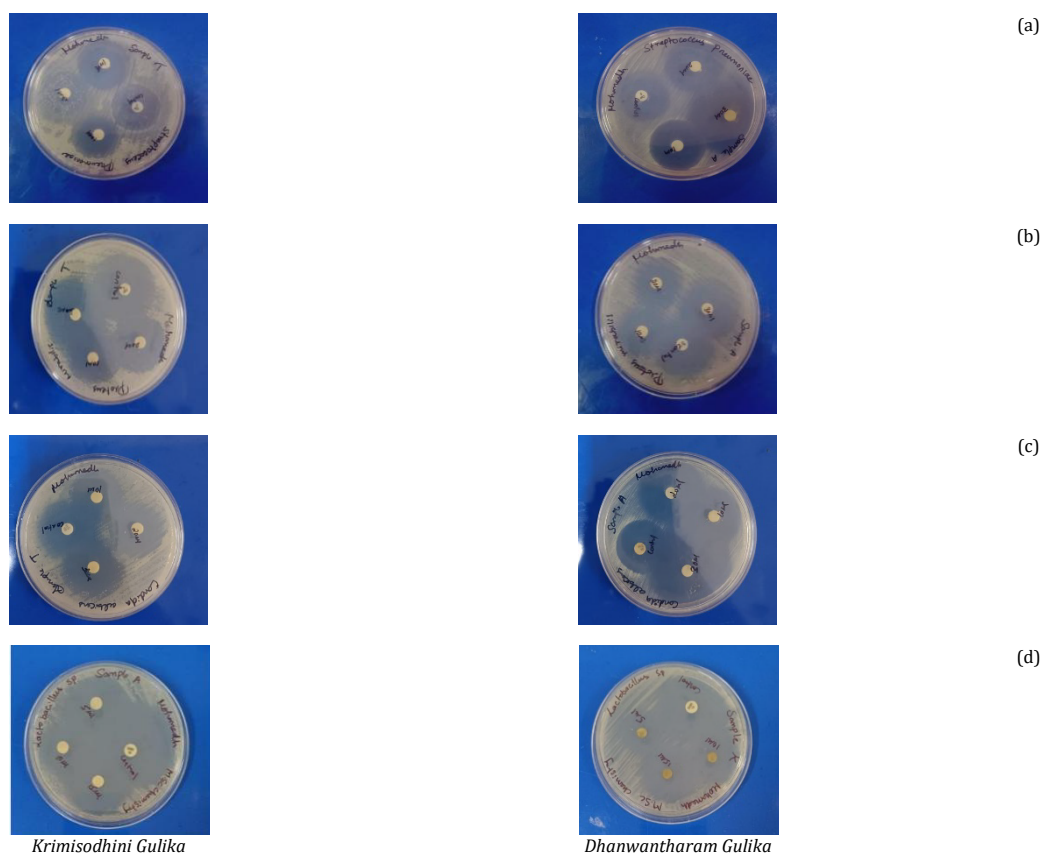
The antimicrobial activity of the Ayurvedic formulations *Krimisodhini Gulika* and *Dhanwantharam Gulika* was evaluated using the agar disc diffusion method against selected bacterial and fungal pathogens, including *Streptococcus pneumoniae*, *Proteus mirabilis*, *Lactobacillus acidophilus*, and *Candida albicans*. Standard antibiotics, namely ciprofloxacin and fluconazole, along with a reference drug, were employed as positive controls to validate the assay. The antimicrobial activity was assessed by measuring the diameter of the zones of inhibition (mm), reflecting the susceptibility of the test organisms to the formulations.

3.9.2. Results and interpretation

The results presented in Table 5 indicate that both formulations produced measurable zones of inhibition against several of the tested microorganisms.

Table 5. In vitro antimicrobial activity of *Krimisodhini Gulika* and *Dhanwantharam Gulika* against selected microbial strains using the agar disc diffusion method.

Ayurvedic medicines	Test organisms	Standard antibiotic-impregnated disc	Low dose: mean $\pm$ SD (mm; n = 3)		
			10 μ L	20 μ L	30 μ L
<i>Krimisodhini Gulika</i>	<i>Streptococcus pneumoniae</i>	Ciprofloxacin CIP 24.833 $\pm$ 0.562 mm /30 μ L	25.821 $\pm$ 0.464	28.733 $\pm$ 0.321	35.967 $\pm$ 0.250
	<i>Proteus mirabilis</i>	Ciprofloxacin CIP 28.933 $\pm$ 0.602 mm / 30 μ L	19.033 $\pm$ 0.45	22.033 $\pm$ 0.650	27.933 $\pm$ 0.602
	<i>Candida albicans</i>	Fluconazole FLU 28.00 $\pm$ 0.50 mm /30 μ L	20.032 $\pm$ 0.654	30.367 $\pm$ 0.484	37.167 $\pm$ 0.364
<i>Dhanwantharam Gulika</i>	<i>Streptococcus pneumoniae</i>	Ciprofloxacin CIP 25.367 $\pm$ 0.473 mm /30 μ L	26.233 $\pm$ 0.168	29.733 $\pm$ 0.222	35.967 $\pm$ 0.251
	<i>Proteus mirabilis</i>	Ciprofloxacin CIP 29.000 $\pm$ 0.612 / 30 μ L	23.367 $\pm$ 0.401	22.033 $\pm$ 0.453	28.133 $\pm$ 0.511
	<i>Candida albicans</i>	Fluconazole FLU 28.067 $\pm$ 0.403 mm /30 μ L	20.033 $\pm$ 0.178	23.233 $\pm$ 0.777	28.142 $\pm$ 0.321
Ayurvedic medicines	Test organisms	Standard antibiotic-impregnated disc	5 μ L	10 μ L	15 μ L
<i>Krimisodhini Gulika</i>	<i>Lactobacillus acidophilus</i>	Doripenem DOR 38.167 $\pm$ 0.364 mm /15 μ L	15.100 $\pm$ 0.252	24.967 $\pm$ 0.650	35.067 $\pm$ 0.502
<i>Dhanwantharam Gulika</i>	<i>Lactobacillus acidophilus</i>	Doripenem DOR 37.967 $\pm$ 0.451 mm / 15 μ L	19.433 $\pm$ 0.192	28.023 $\pm$ 0.351	35.400 $\pm$ 0.654

**Figure 2.** Representative paired agar disc-diffusion assay plates for *Krimisodhini Gulika* (left) and *Dhanwantharam Gulika* (right) against (a) *Streptococcus pneumoniae*, (b) *Proteus mirabilis*, (c) *Candida albicans*, and (d) *Lactobacillus acidophilus*, with the corresponding standard controls.

Krimisodhini Gulika showed larger inhibition zones against *Candida albicans* and *Streptococcus pneumoniae* under the tested conditions, whereas *Dhanwantharam Gulika* also exhibited inhibition against the tested bacterial and fungal strains. The magnitude of inhibition varied with formulation, microorganism and tested concentration. These findings demonstrate in vitro antimicrobial activity under the experimental conditions used; however, direct comparisons with standard antimicrobial agents should be interpreted cautiously unless supported by appropriate statistical testing and comparable dosing conditions. Representative agar disc-diffusion assay plates showing the antimicrobial activity of *Krimisodhini Gulika* and *Dhanwantharam Gulika* against the tested microorganisms are presented in Figure 2. The solvent-

treated negative-control discs produced no measurable zones of inhibition under the test conditions.

3.9.3. Possible mechanistic interpretation of antimicrobial activity

The antimicrobial activity observed in the present study may reflect the combined effects of multiple constituents present in the formulations. Several classes of compounds tentatively assigned by GC-MS, including terpenoid-related compounds, fatty acids and other secondary metabolites, have been reported in the literature to interact with microbial membranes, metabolic pathways or other cellular targets [31,33,35]. These reported mechanisms provide possible

explanations for the antimicrobial activity observed in the present formulation-level assays. However, the present study did not investigate the mechanism of action of individual constituents or determine whether interactions among constituents were synergistic, additive or antagonistic. Therefore, these mechanisms should be regarded as plausible literature-based hypotheses rather than experimentally demonstrated mechanisms of the formulations. Further studies using purified constituents and appropriate mechanistic and synergy assays are required to establish the specific contributions of individual compounds and their interactions.

3.9.4. Interpretation of antimicrobial findings

Both *Krimisodhini Gulika* and *Dhanwantharam Gulika* demonstrated in vitro antimicrobial activity against selected bacterial and fungal strains under the conditions examined. The observed inhibition varied among microorganisms and formulation concentrations. These findings provide experimental evidence of antimicrobial activity at the formulation level, but they do not establish the contribution of individual GC-MS-tentatively assigned constituents or demonstrate synergistic interactions among them [7]. The results provide a basis for further investigation of these formulations and their constituent compounds, including quantitative chemical characterization, compound-specific antimicrobial assays and appropriate combination studies.

3.9.5. Molecular docking results and discussion

The molecular docking analysis was performed to explore the potential interactions of tentatively assigned candidate compounds from the Ayurvedic formulations *Dhanwantharam Gulika* and *Krimisodhini Gulika* with the SiteMap-predicted, non-catalytic surface pocket of gastric lipase. This analysis was undertaken as an exploratory and secondary hypothesis, rather than as evidence that gastric lipase represents a validated therapeutic target of these formulations. The primary focus of the present study remains the antimicrobial potential of the formulations and their tentatively assigned candidate compounds. However, considering the traditional use of these formulations and previously reported pharmacological activities [30,32] of their constituent phytochemicals, the possibility of additional biological effects was considered and investigated computationally. Thus, gastric lipase was selected as a complementary target to examine whether some of the tentatively assigned candidate compounds could potentially interact with an enzyme involved in gastrointestinal lipid metabolism [18]. The docking scores and MM-GBSA binding free energies were used as comparative parameters for ranking the predicted ligand-protein interactions within the screened compound set rather than as absolute measures of binding affinity. The Glide docking scores obtained in the present study ranged from approximately -2.8 to -4.1 kcal/mol, indicating relatively modest predicted interactions rather than strong binding. Compounds showing comparatively more negative docking scores and MM-GBSA binding free energies than the other screened constituents and standard/control compounds were considered to exhibit relatively favourable predicted interactions within the present dataset and were therefore prioritised for subsequent pharmacokinetic evaluation [8]. Experimental validation, particularly through gastric lipase enzyme inhibition assays and appropriate biochemical studies, will be necessary to determine whether the predicted interactions correspond to measurable biological activity.

3.9.6. Docking analysis of *Krimisodhini Gulika*

The docking results revealed that several phyto-constituents from *Krimisodhini Gulika* exhibited detectable,

moderate binding affinity towards the SiteMap-predicted gastric-lipase surface pocket. Among the screened compounds, 3-hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl- (K18b) showed the most favourable docking score within this dataset (-4.074 kcal/mol) along with a comparatively favourable MM-GBSA binding energy of -27.61 kcal/mol, indicating a relatively more stable, though still modest, predicted ligand-protein interaction. Similarly, ethanol, 2-(9,12-octadecadienyloxy)- (K14c) demonstrated a docking score of -4.034 kcal/mol and binding energy of -22.14 kcal/mol, consistent with moderate hydrophobic interactions within the SiteMap-predicted, non-catalytic surface pocket rather than high-affinity binding. Other notable compounds, such as L- α -terpineol (K4c) and α -terpineol (K4a/K4b), also exhibited similar docking scores (~-3.85 kcal/mol) with corresponding MM-GBSA estimates; their terpene-based lipophilic structures may support predicted contacts with hydrophobic residues [8]. Additionally, fatty acid derivatives like 9-octadecenoic acid ester (K16c) and conjugated linoleic acid derivatives (K12a, K12b) showed relatively strong MM-GBSA energies (up to -31.57 kcal/mol), providing comparatively more favourable MM-GBSA estimates within the applied computational model despite moderate docking scores. Interestingly, some compounds, such as pyrimidine-4,6-diol (K1c) exhibited less favourable or even positive MM-GBSA values, suggesting weaker or unstable binding interactions within the SiteMap-predicted surface pocket. The results suggest that terpenoids and long-chain fatty acid derivatives may contribute to the predicted interaction profile of *Krimisodhini Gulika*. Representative molecular docking interactions of selected *Krimisodhini Gulika* constituents with gastric lipase are shown in Figure 3.

3.9.7. Docking analysis of *Dhanwantharam Gulika*

The docking study of *Dhanwantharam Gulika* indicated detectable interactions between its bioactive constituents and gastric lipase, with an overall binding profile of similarly modest magnitude to that observed for *Krimisodhini Gulika*. The identified compounds predominantly interacted with the SiteMap-predicted non-catalytic surface pocket through hydrophobic and van der Waals interactions. Among the identified constituents, isoborneol (D2a/D2c) and endoborneol (D2b) showed docking scores of approximately -3.077 kcal/mol, with MM-GBSA binding energies of around -15.82 kcal/mol, suggesting relatively stable, although weak-to-moderate, predicted interactions within the identified surface pocket. Isobornyl formate (D3a) exhibited docking scores ranging from -3.351 to -2.844 kcal/mol, indicating variable predicted binding depending on the evaluated pose or conformational state, while its corresponding MM-GBSA values suggested moderate binding affinity. In contrast, camphor (D1b) and bornyl chloride (D3b) demonstrated comparatively less favourable binding profiles, consistent with weaker interactions with residues lining the predicted surface pocket [36]. Representative molecular docking interactions of selected *Dhanwantharam Gulika* constituents with gastric lipase are shown in Figure 4.

3.9.8. Comparative docking assessment

Within the applied computational model, some *Krimisodhini Gulika* constituents produced more negative docking scores and MM-GBSA estimates than the screened *Dhanwantharam Gulika* constituents, although the predicted interactions remained moderate. These differences may reflect variation in ligand structure and predicted interaction patterns [8]; however, they should not be interpreted as evidence of enhanced biological activity or therapeutic efficacy.

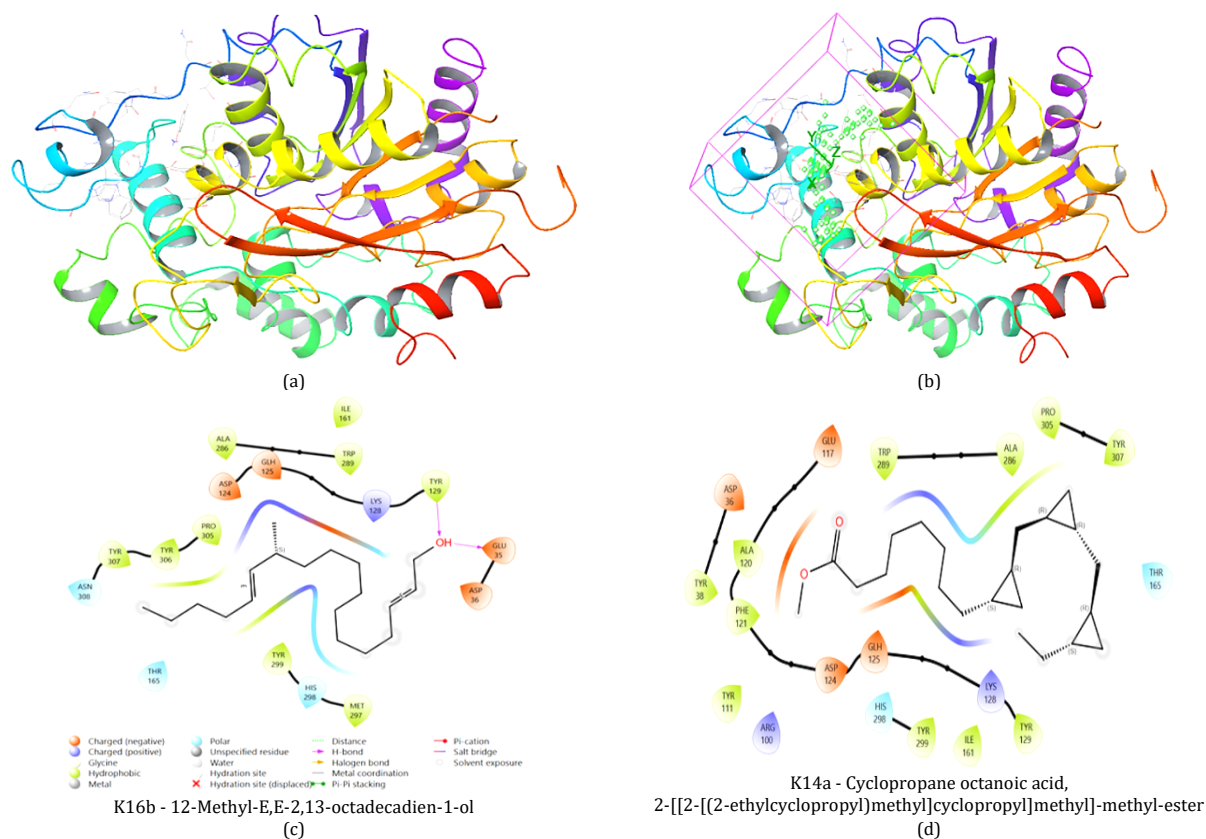


Figure 3. Molecular docking interactions of selected tentatively identified phytoconstituents from *Krimisodhini Gulika* within the SiteMap-predicted, non-catalytic surface pocket of gastric lipase.

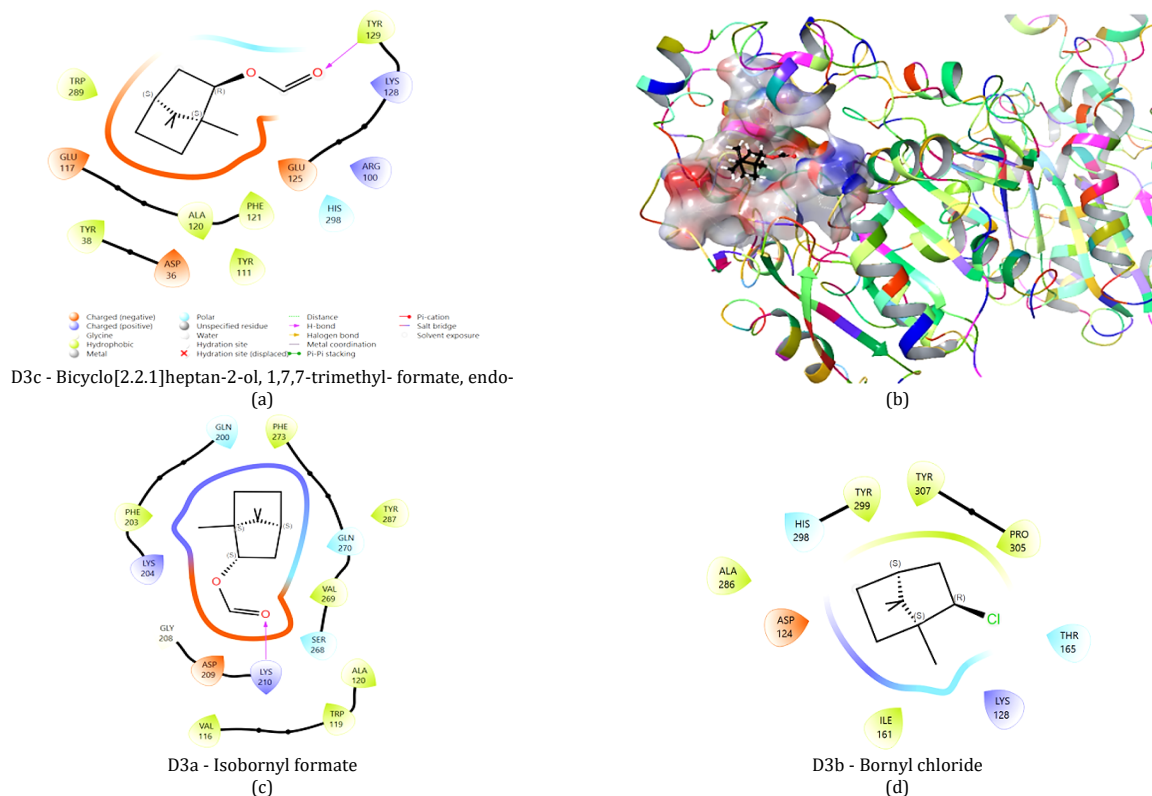


Figure 4. Molecular docking interactions of selected tentatively identified phytoconstituents from *Dhanwantharam Gulika* within the SiteMap-predicted, non-catalytic surface pocket of gastric lipase.

Table 6. Predicted QikProp ADMET descriptors for the GC-MS library candidates from *Krimisodhini Gulika* included in the QikProp analysis. QPlogPo/w, predicted octanol/water partition coefficient; QPlogS, predicted aqueous solubility; QPPCaco, predicted apparent Caco-2 cell permeability; QPlogBB, predicted brain/blood partition coefficient; QPlogKhsa, predicted binding to human serum albumin.

Compound code	Mol. weight (g/mol)	QPlogPo/w	QPlogS	QPPCaco (nm/s)	QPlogBB	QPlogKhsa	Predicted human oral absorption (%)	#metab	Rule-of-Five violations
K1a	126.1	0.75	-0.79	1060.0	-0.26	-0.70	85.5	2	0
K1b	168.1	0.18	-0.35	1557.1	-0.17	-1.09	85.1	1	0
K1c	126.1	0.30	-0.42	414.5	-0.65	-0.66	75.6	4	0
K2a	152.2	1.93	-1.99	4109.1	0.27	-0.19	100.0	1	0
K2b	152.2	1.93	-1.99	4085.1	0.26	-0.18	100.0	1	0
K2c	152.2	1.94	-1.99	4109.9	0.27	-0.18	100.0	1	0
K3a	154.3	2.05	-2.08	4237.2	0.21	-0.11	100.0	1	0
K3b	154.3	2.05	-2.07	4236.7	0.22	-0.11	100.0	1	0
K3c	154.3	2.05	-2.08	4237.2	0.21	-0.11	100.0	1	0
K4a	154.3	2.94	-2.65	4652.7	0.17	0.11	100.0	4	0
K4b	154.3	2.94	-2.65	4652.7	0.17	0.11	100.0	4	0
K4c	154.3	2.94	-2.64	4681.5	0.17	0.11	100.0	4	0
K5a	136.2	2.16	-1.74	267.1	-0.36	-0.58	83.0	1	0
K5b	159.2	1.24	-1.92	1170.8	-0.45	-0.71	89.1	2	0
K5c	180.2	2.08	-1.51	12.8	-0.98	-0.68	58.9	2	0
K6a	150.2	1.84	-1.44	236.7	-0.50	-0.47	80.2	2	0
K7a	172.3	2.94	-2.86	234.6	-0.93	-0.21	86.6	1	0
K7b	172.3	2.87	-2.61	236.0	-0.84	-0.19	86.2	1	0
K7c	186.3	3.31	-3.30	237.8	-1.01	-0.10	88.8	1	0
K8a	200.3	3.70	-3.76	235.5	-1.11	0.03	91.1	1	0
K8b	214.3	4.08	-4.19	236.3	-1.20	0.15	93.3	1	0
K8c	242.4	4.83	-5.03	235.3	-1.38	0.39	100.0	1	0
K9a	198.3	2.87	-3.38	2199.7	-0.53	-0.03	100.0	1	0
K9b	226.4	3.60	-4.15	2194.1	-0.68	0.21	100.0	1	0
K9c	184.3	2.49	-2.93	2197.8	-0.44	-0.16	100.0	1	0
K10a	228.4	4.44	-4.55	239.8	-1.28	0.26	95.5	1	0
K10b	242.4	4.83	-5.03	236.1	-1.38	0.39	100.0	1	0
K10c	214.3	4.07	-4.17	236.3	-1.20	0.15	93.3	1	0
K11a	256.4	5.20	-5.43	236.9	-1.47	0.51	86.9	1	1
K11b	653.0	8.74	-8.58	342.9	-3.49	1.52	100.0	5	2
K11c	494.8	9.88	-10.94	1600.8	-2.32	2.17	100.0	2	1
K12a	280.4	5.87	-6.51	235.7	-1.54	0.78	90.8	3	1
K12b	280.4	5.75	-6.22	235.1	-1.51	0.73	90.1	3	1
K12c	280.4	5.85	-6.47	237.0	-1.53	0.78	90.8	4	1
K13a	280.4	5.76	-6.22	235.9	-1.51	0.73	90.2	4	1
K13b	280.4	5.69	-5.90	236.8	-1.65	0.60	89.8	2	1
K13c	280.4	1.24	2.00	3399.5	-0.51	-1.20	100.0	3	0
K14a	334.5	6.98	-8.12	3004.2	-0.86	1.55	100.0	1	1
K14b	280.4	5.99	-6.70	3911.8	-0.70	1.06	100.0	4	1
K14c	310.5	6.12	-7.00	3027.2	-1.20	0.92	100.0	5	1
K15a	268.4	5.05	-5.19	2975.0	-0.72	0.70	100.0	1	1
K15b	280.5	6.14	-6.88	2824.3	-1.01	1.11	100.0	4	1
K15c	268.4	5.56	-6.30	3053.8	-0.76	0.96	100.0	2	1
K16a	268.4	5.05	-5.22	2984.1	-0.72	0.70	100.0	1	1
K16b	280.5	6.14	-6.88	2828.7	-1.01	1.10	100.0	4	1
K16c	338.5	5.83	-5.43	4549.9	-0.84	0.70	100.0	3	1
K17a	392.7	7.80	-7.56	4481.4	-0.71	1.77	100.0	1	1
K17b	418.7	9.21	-9.98	3568.2	-1.23	2.20	100.0	9	1
K17c	308.5	5.91	-6.58	3931.8	-0.41	1.21	100.0	1	1
K18a	278.4	3.37	-3.65	3690.8	-0.01	0.14	100.0	3	0
K18b	236.4	4.19	-4.53	3420.4	-0.23	0.62	100.0	7	0
K18c	235.2	2.82	-3.04	4094.8	0.27	0.09	100.0	1	0

In contrast, *Dhanwantharam Gulika* predominantly contains monoterpenoid derivatives, which, although capable of binding to the enzyme, exhibit comparatively moderate affinity. The presence of multiple constituents with predicted interactions may provide hypotheses for future investigation of possible multi-component effects; however, synergistic pharmacological activity was not evaluated in the present study.

3.9.9. Study implications and limitations

The docking results suggest that both Ayurvedic formulations contain bioactive constituents capable of moderate, non-covalent interaction with gastric lipase, providing preliminary in silico support for their suitability for further computational and experimental evaluation; given the modest docking score range observed, these findings should be regarded as hypothesis-generating rather than conclusive evidence of strong binding, and warrant confirmation by in vitro enzyme inhibition assays. Compounds with comparatively

more favourable docking profiles were further subjected to in silico ADMET profiling using the QikProp module (Schrödinger Release 2024-2: QikProp, Schrödinger, LLC) [37] to evaluate their drug-likeness and pharmacokinetic properties, as summarised in Table 6. The study provides preliminary computational data for future investigation of gastric-lipase-related hypotheses.

3.10. In silico ADMET profiling of *Krimisodhini Gulika* constituents

Pharmacokinetic and drug-likeness properties were predicted for 52 of the 54 GC-MS library candidates reported for *Krimisodhini Gulika* using the QikProp module (Schrödinger Release 2024-2: QikProp, Schrödinger, LLC) [37]; this candidate set includes alternative spectral matches and should not be interpreted as 52 independently detected constituents. The available QikProp dataset contained 52 entries; K6b (benzenepropanoic acid, silver(1+) salt) and K6c (4-nitrosophenyl-β-phenylpropionate) were not included in Table

6 and are therefore excluded from the ADMET interpretation. The GC-MS dataset therefore contains 54 library candidates associated with 18 chromatographic peaks. Of the full descriptor set generated, the pharmacokinetically most informative parameters are summarised in Table 6, comprising predicted molecular weight, octanol/water partition coefficient (QPlogPo/w), aqueous solubility (QPlogS), apparent Caco-2 cell permeability (QPPCaco), blood-brain barrier partition coefficient (QPlogBB), serum albumin binding (QPlogKhsa), percentage human oral absorption, the predicted number of possible metabolic reactions (#metab), and the number of Lipinski Rule-of-Five violations. Most candidates fell within the recommended ranges for the evaluated QikProp descriptors (mol. wt. 130–725; QPlogPo/w –2.0 to 6.5; QPlogS –6.5 to 0.5; QPPCaco >25 nm/s; QPlogBB –3.0 to 1.2; QPlogKhsa –1.5 to 1.5; and predicted human oral absorption >80% considered high). Candidates with no more than one Lipinski Rule-of-Five violation were considered broadly consistent with the Rule-of-Five framework. However, these computational criteria alone do not establish oral bioavailability, pharmacological safety or drug suitability.

Mol. Wt., predicted molecular weight (g/mol); QPlogPo/w, predicted octanol/water partition coefficient; QPlogS, predicted aqueous solubility; QPPCaco, predicted apparent Caco-2 cell permeability (nm/s); QPlogBB, predicted blood-brain barrier partition coefficient; QPlogKhsa, predicted binding to human serum albumin; #metab, predicted number of possible metabolic reactions; RoF viol., number of Lipinski Rule-of-Five violations. Compound codes correspond to those listed in Table 3. The phytoconstituents showing comparatively favourable docking profiles were further evaluated using in silico ADMET analysis [37] to assess their predicted drug-likeness and pharmacokinetic properties. The analysis included physicochemical and pharmacokinetic parameters such as molecular weight, hydrogen-bonding properties, lipophilicity, aqueous solubility, gastrointestinal absorption, and other relevant descriptors [37]. The selected constituents showed variable predicted pharmacokinetic profiles, with several compounds displaying acceptable drug-like characteristics. These computational predictions provide preliminary information for prioritizing compounds for further experimental investigation but should not be interpreted as evidence of pharmacological safety or efficacy.

4. Conclusion and future perspectives

The present study provided an integrated phytochemical, spectroscopic, antimicrobial, molecular docking, and in silico ADMET evaluation of the Ayurvedic formulations *Dhanwantharam Gulika* and *Krimisodhini Gulika*. GC-MS analysis revealed distinct and chemically diverse profiles in the two formulations, with the detected constituents reported as tentative library-based assignments. FTIR, UV-Vis, and ¹H NMR analyses further supported the presence of diverse chemical functionalities and structural features. Both formulations demonstrated antimicrobial activity against the tested bacterial and fungal strains under the experimental conditions employed, with *Krimisodhini Gulika* producing larger inhibition zones against some tested microorganisms under the experimental conditions. The GC-MS findings provide a basis for further investigation of the detected constituents; however, they do not establish that any individual compound is responsible for the observed antimicrobial activity or demonstrate synergistic interactions among constituents. Similarly, the molecular docking and ADMET results represent preliminary computational predictions and do not provide direct evidence of enzyme inhibition, bioavailability, pharmacological efficacy, or therapeutic benefit. Further studies are warranted to quantitatively characterize the major constituents and confirm their structures using appropriate analytical

standards. Bioassay-guided fractionation, isolation and individual compound testing, together with defined combination and synergy assays, are needed to determine the contribution and possible interactions of individual constituents to the observed antimicrobial activity. Experimental enzyme-inhibition studies are also required to validate the molecular docking predictions, while pharmacokinetic, toxicological and appropriate in vivo investigations are necessary to evaluate the biological and pharmacological relevance of the formulations.

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CRediT authorship contribution statement

Conceptualization: Mohamed Ahadu Shareef; Methodology: Mohamed Zubair; Software: Mohamed Divan Masood; Validation: Mohamed Zubair; Formal Analysis: Mohamed Divan Masood; Investigation: Irfan Navabshah; Resources: Irfan Navabshah; Data Curation: Irfan Navabshah; Writing - Original Draft: Mohamed Ahadu Shareef; Writing - Review and Editing: Mohamed Zubair; Visualization: Mohamed Divan Masood; Funding acquisition: Mohamed Ahadu Shareef; Supervision: Mohamed Ahadu Shareef; Project Administration: Mohamed Ahadu Shareef.

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ORCID and Email

Mohamed Ahadu Shareef

 ahadushareef@thenewcollege.edu.in

 jasshaali@gmail.com

 <https://orcid.org/0000-0002-1619-7042>

Mohamed Zubair

 mohamedzubair8695@gmail.com

 <https://orcid.org/0009-0000-0449-8035>

Irfan Navabshah

 nirfanahamed1985@live.com

 <https://orcid.org/0000-0003-4630-5259>

Mohamed Divan Masood

 divan@crescent.education

 divan.masood77@gmail.com

 <https://orcid.org/0000-0002-9396-3740>

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