ORIGINAL ARTICLE

In Silico Screening of Potent Bioactive Compounds from *Syzygium aromaticum* against Drug-Resistant Oral Pathogens

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ABSTRACT

Introduction: With antibiotic-resistant infections projected to exceed 10 million annually, identifying novel antimicrobial agents is critical. This study investigates the protein-ligand interactions of bioactive components from *Syzygium aromaticum* (clove) against resistant oral pathogens. **Methods:** Clove samples characterized by Fourier-transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), and phytochemicals analysis were performed to detect bioactive compounds. The antioxidant activity of clove was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The computational study used the identified ligands and proteins of oral pathogens. **Results:** FTIR and GC-MS identified key functional groups, such as hydroxyl groups and aromatic rings, confirming the presence of phenolic substances like eugenol and β-caryophyllene. The DPPH assay demonstrated robust antioxidant activity across varying doses. Specific compounds, including 2-tert-butylphenol and trimethylsilyl ether, were identified for their potential to enhance bioavailability and membrane permeability. Furthermore, ADME analysis of eugenol revealed superior gastrointestinal absorption, blood-brain barrier penetration, and favorable pharmacokinetic profiles. **Conclusion:** Collectively, these results confirm the therapeutic potential of clove, namely, its antioxidant, antibacterial, and anti-inflammatory properties, emphasizing its suitability for medical and pharmaceutical applications.
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Keywords: Molecular docking, *Syzygium aromaticum*, plant extract, protein-ligand inhibition, oral pathogens.

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INTRODUCTION

In recent years, there has been an increase in reports on the establishment of resistant strains, especially those that develop resistance to many medications. Furthermore, it has been shown that these resistant phenotypes can emerge during illness and in response to therapy, adding further risks to patients (1). Treatment options for bacteria with high antibiotic resistance rates, such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterococcus faecium* (ESKAPE), are decreasing (2). Extra risk factors often include conditions like immune suppression, old age, admission to intensive care units, mechanical ventilation, solid organ or hematopoietic transplantation, or extended hospital stays that enhance exposure to antibiotic-resistant microorganisms. Undoubtedly, the use of antibiotics in the past increased the risk (3).

Antimicrobial resistance has been rapidly emerging in tandem with the usage of antibiotics in recent years. Antibiotic resistance is a result of over-prescription and inappropriate use of antibiotics in dental illnesses, as reported by many researchers (4). *A. baumannii*, a potentially nosocomial pathogen that, infiltrated the oral cavity covertly, was linked to *P. aeruginosa* in aggressive periodontitis cases. Resistance to endodontic and periodontal therapies is due to its ability to develop biofilms and withstand desiccation, which can lead to infection (5). The renewed focus on identifying novel antibacterial medications against multidrug-resistance (MDR) bacteria, along with numerous recent financing mechanisms, has contributed to increasing the number of classic and atypical antibacterial agents progressing through preclinical and clinical development pipelines (6).

Syzygium aromaticum (clove), a traditional spice used for food preservation, contains numerous phytochemicals, including sesquiterpenes, monoterpenes, hydrocarbons, and phenolic compounds. Clove oil, which contains eugenol acetate, eugenol, and β-caryophyllene, has been studied for its pharmacological properties against pathogenic parasites and microorganisms. Clove essential oil is traditionally used for treating burns and, wounds, pain relief during dental care, and for treating tooth infections and toothaches (7,8). The predominant active components of the clove dichloromethane extract determined, by gas chromatography-mass chromatography (GC-MS) analysis were eugenol and eugenol acetate (9).

S. aromaticum essential oil, extracted through hydrodistillation, exhibits antibacterial properties against food spoilage and pathogenic bacteria and, with radical scavenging activity. Moreover, it increases cell permeabilization and membrane integrity, thereby potentially treating colonic cancers (10). This study confirmed the antibacterial activity of the tested plant compounds, excluding kaempferol and quercetin. Flavonoids and organic acids exert biological effects against 3-4 bacterial species, with greater potency against Gram-negative bacteria. The study found moderate or low flavonoid and organic acid activity compared with traditional antibiotics and some plant substances (11). Another study found that alkaloids of Allium sativum and Bunium persicum showed strong bacterial inhibition zones against various bacterial strains, with alkaloids showing the highest inhibition zones against K. pneumoniae ATCC and S. aureus MDR. Saponins have the highest inhibitory activities against E. coli and crude extract against S. aureus MDR (12).

Advanced computational biology approaches are promising for drug discovery to combat multidrug resistance in bacterial strains. Techniques include virtual screening, molecular docking, MD simulation, quantitative structure-activity relationships (QSAR), and pharmacophore modeling, along with structural genomics (13). Antibiotic resistance proteins were identified using ARG-ANNOT, and three were identified as resistant to antibiotics. These proteins represent potential drug targets for the treatment of infectious diseases (14). Antibiotic resistance (ABR) is a major oral microbiome disorder causing antibiotic-resistant infections. Identifying ABR genes is challenging, but Whole-Genome Sequencing and metagenomics can help characterize ABR and improve treatment efficiency (15).

MATERIALS AND METHODS

Preparation of aqueous extract

S. aromaticum buds were collected from a local vendor in Chennai, Tamil Nadu, India. The samples were authenticated by the Center for Advanced Studies in Botany at the University of Madras, Chennai, India. The clove is made into a powder by various processes. The clove aqueous extract was prepared using powder (25 g) and distilled water (200 mL). The clove bud extract was stored at 4°C for further experiments (16).

Characterization of clove

After the extraction process, the clove was analyzed using Fourier transform infrared (FTIR) spectroscopy in the range of 4000-400 cm-1. This analysis effectively identified functional groups present in the clove.

GC-MS analysis was conducted using 100 µL of an aqueous solution of clove dissolved in 1 mm of methanol. The solution was agitated firmly using a vortex stirrer for 20 s and then filtered through a 0.2-m membrane filter. Subsequently, this clear extract was used for GC-MS examination. The method was outlined by Rao et al., (17).

Phytochemical analysis

The aqueous extract of clove was subjected to qualitative phytochemical analysis to investigate the existence of different bioactive components. The analysis comprised a sequence of conventional chemical screenings designed to identify particular categories of phytochemicals. The subsequent protocols were implemented following previous research (18).

Antioxidant assay

The antioxidant assay of clove was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. This assay was based on the protocol described by Behera et al., (18). This test quantifies the clove extract's capacity to donate hydrogen atoms or electrons to neutralize DPPH, a volatile free radical, resulting in the formation of a colorless or bleached product that can be measured by spectrophotometry.

In silico study

The protein structures were acquired from RCSB PDB and verified using a build/check/repair model to confirm their integrity. Auto Dock Tools were used to prepare the pdbqt files. The ligands obtained from PubChem were subjected to optimization using the Avogadro software and were subsequently converted for docking. AutoDock4 performed molecular docking using a refined grid, followed by cluster analysis to identify the most favorable binding positions. The binding interactions and affinities were determined using Biovia Discovery Studio. Swiss ADME was used to examine *in silico* Absorption Distribution Metabolism Excretion (ADME) research, which involved evaluating drug-likeness according to Lipinski's rule of five and predicting therapeutic efficacy and safety by examining interactions with biological components (17) (Fig. 1).

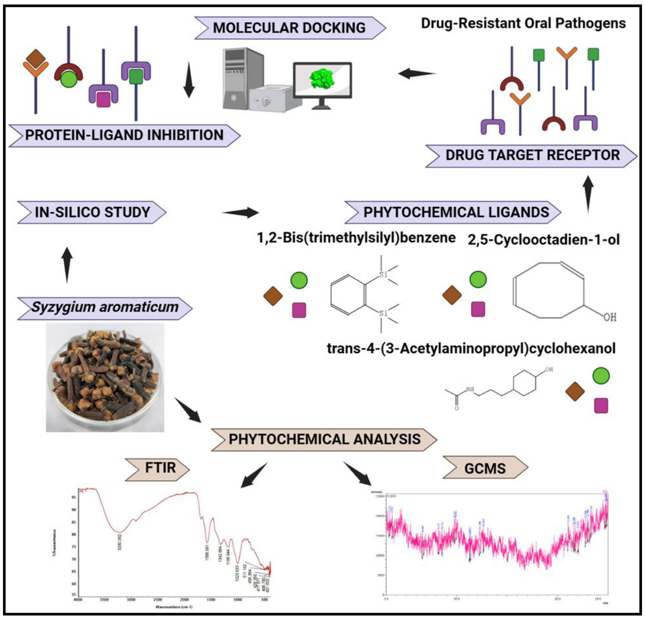


Figure 1: Graphical abstract of the identification of bioactive compounds in *Syzygium aromaticum*.

RESULTS

FTIR

The clove exhibits important functional groups in its FTIR spectrum, as shown in Fig. 2, which indicates its bioactive components. O-H stretching is indicated by the large peak at 3230.062 cm^{-1}, which points to the hydroxyl groups that are characteristic of phenolic substances. The presence of aromatic rings is confirmed by the peak at 1588.991 cm^{-1}, which corresponds to C=C stretching. C-N and C-O stretching are represented by the peaks at 1342.884 cm^{-1} and 1196.944 cm^{-1}, respectively, indicating the existence of amines, alcohols, ethers, or esters. Subsequent peaks located below 1500 cm^{-1} in the fingerprint region intricate molecular architecture. These results highlight the existence of aromatic rings and polyphenolic chemicals, which support clove's powerful biological effects and health advantages.

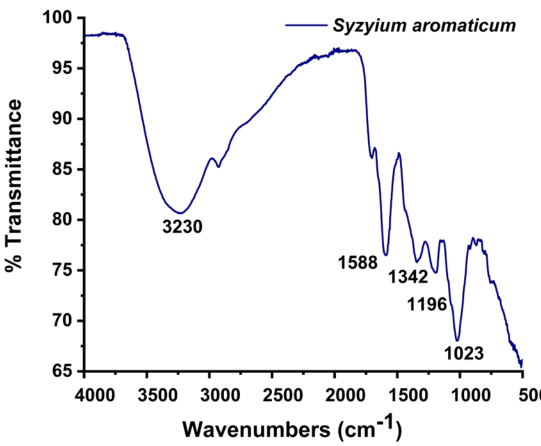


Figure 2: FTIR spectrum of the *Syzygium aromaticum* extract

GCMS

Significant peaks with retention durations of 5.610, 6.015, 8.544, 13.200, 14.843, 21.060, and 23.235 min are revealed in Fig. 3, GC-MS analysis of clove reveals, the presence of several bioactive chemicals. Given its antibacterial and anti-inflammatory properties, eugenol, the main ingredient in clove oil, is most likely responsible for the peak at 8.544 min. The presence of β-caryophyllene, a molecule with analgesic and anti-inflammatory properties, was indicated by the peak observed at 13.200 min. Clove's medicinal qualities of clove may enhanced by humulene or other sesquiterpenes, as indicated by the peak at 21.060 min. These results complement historical data.

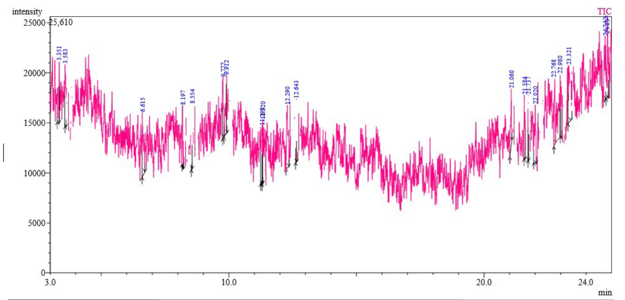


Figure 3: GC-MS chromatogram of the *Syzygium aromaticum* extract

Phytochemical test

Clove has a rich profile with great medicinal potential, as shown in the phytochemical research. Significant antioxidant and anti-inflammatory activities are indicated by flavonoids (+) and terpenoids (+). High concentratiosn of phenols (+++) and tannins (+++) indicate strong astringent and antibacterial properties. The presence of fatty acids (++) indicates their contribution to heart health and anti-inflammatory effects, whereas the detection of carbs (+) indicates potential energy-providing and prebiotic advantages. Alkaloids, saponins, glycosides, and proteins are conspicuously lacking, indicating a particular phytochemical composition that could impact clove's overall therapeutic uses. The presence of such a wide range of phytochemicals highlights the many applications of clove in medicine.

Antioxidant assay

The substantial antioxidant activity of the bioactive components of clove is shown by the DPPH assay results in Fig. 4. From 10 to 50 µg/mL, the proportion of inhibition increased with increasing concentration. The clove showed almost 75% inhibition at 10 µg/mL, which is in close agreement with the standard. Clove continuously exhibits high inhibition, comparable to that of the standard, at all concentrations; this pattern is especially evident at higher concentrations (40 and 50 µg/mL). Minimal experimental variances are suggested by the small error bars. These results demonstrate clove's potential as a natural antioxidant source by demonstrating

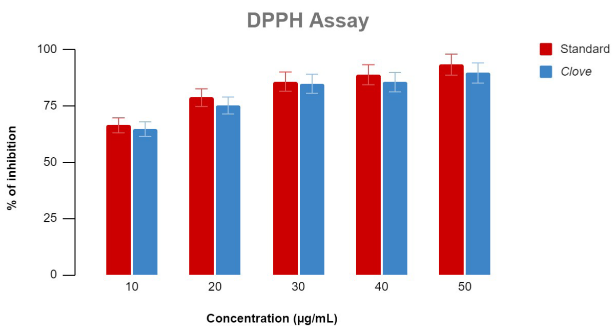


Figure 4: DPPH assay of the *Syzygium aromaticum* extract

that it possesses strong antioxidant qualities that are on par with those of traditional antioxidants.

Molecular docking analysis

Some bioactive chemicals listed in Table I and II are 2-tert-Butylphenol, trimethylsily ether, 1,2-Bis(trimethylsilyl) benzene, 2,5-Cyclooctadien-1-ol, and other chemicals associated with clove. They also have numerous forms of visualization, especially in two- and three-dimensional molecular spaces. There is a possibility that every component of the mixture imparts certain characteristics to the total bioactivity of the extract. For instance, 2,5-Cyclooctadien-1-ol may enhance bioavailability and metabolic stability, whereas 1,2-Bis(trimethylsilyl) benzene may enhance stability. This suggests an increase in lipophilicity and membrane permeability due to 2-tert-Butylphenol and trimethylsily ether. These interactions indicate that clove has numerous medical uses. They are easily synthesized and have moderate aqueous solubility.

ADME Properties of Drug

The molecule depicted in Fig. 5 is eugenol, a bioactive compound derived from clove. The ADME radar plot reveals several important characteristics: Regarding the chemical certainty, the molecule possesses a moderately large size and appropriate lipophilicity, which probably means that the molecule has acceptable membrane permeability. Thus, the polarity is within the range, which indicates that there is significant solubility and absorption. In contrast, low flexibility and low insolubility represent conformations with poor flexibility and compounds that do not dissolve easily in water. In addition, the increasing stability is low, which shows that there is no tendency toward the presence of double bonds. Eugenol has a fairly good ADME balance, which is suitable for medicinal purposes.

Eugenol, the clove's bioactive component, has a formula of C11H16O2 and a molecular weight of 180.24 g/mol. This SMILES can be represented as CC(C)(C) C1=C(C=CC(=C1) OC) O. Eugenol can pass through the blood-brain barrier (BBB) and exhibits high gastrointestinal (GI) absorption with a topological polar surface area (TPSA) of 29.46 E . It has a bioavailability score of 0.55, indicating moderate oral bioavailability, and no violations of Lipinski's rule of five, indicating good drug-like qualities. With a synthetic accessibility rating of 1.37, the synthesis was easy. The moderate water solubility of eugenol contributes to its potential medicinal uses.

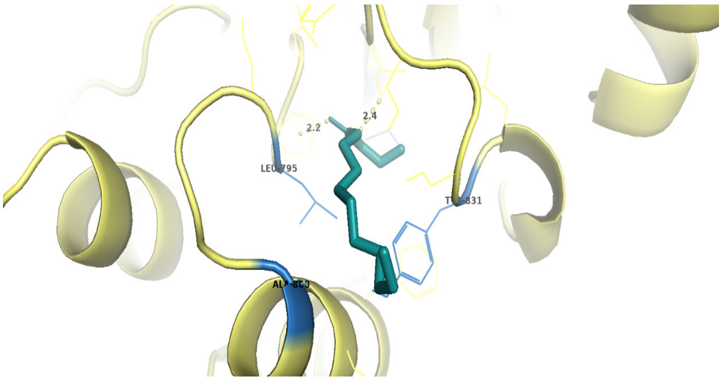
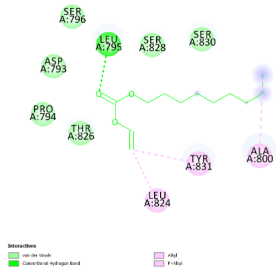
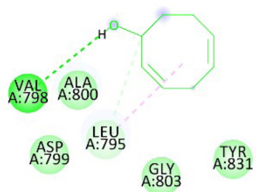
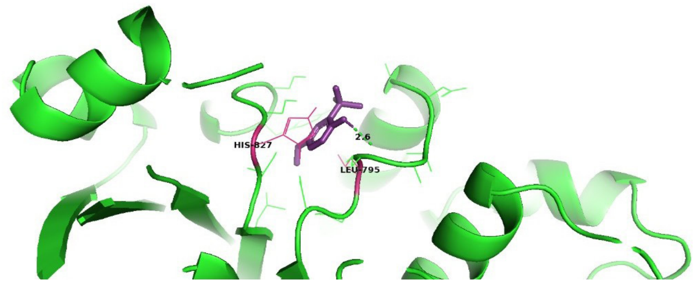
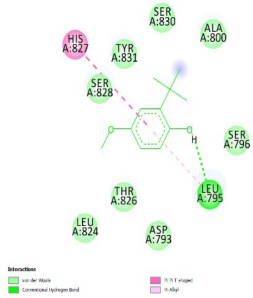
DISCUSSION

The research planned to identify the phytochemical constituents of clove using different methods which were; FTIR, GC-MS, Phytochemical screening tests, antioxidant activities, molecular docking, and ADME. The FTIR analysis identified the functional groups by the bioactive compounds in which the major peaks illustrated the compound containing O-H stretching

Table I: Molecular docking analysis of the *Syzygium aromaticum* extract

Name of the Ligand	Binding Affinity Value	Distance	Hydrogen Interaction	Amino acid residues
1,2-Bis(trimethylsilyl) benzene	**-4.5**	2.4 (TYR 831) 2.2(LEU 795)	1. Van der Waals 2. Conventional Hydrogen Bond 3. Alkyl	1. PRO A:794, THR A:826, ASP A:793, SER A:796, SER A:828, SER A:830 2. LEU A:824, TYR A:831, ALA A:800
2,5-Cyclooctadien-1-ol	**-4.6**	2.8 (VAL 798)	1. Van der Waals 2. Conventional Hydrogen Bond 3. Alkyl	1. ALA A:800, LEU A:795, GLY A:803, TYR A:831, SER A:830 2. VAL A:798 3. LEU A:795
2-tert-Butylphenol, trimethylsily ether	**-5.5**	2.6(LEU 795)	1. Van der Waals 2. Conventional Hydrogen Bond 3. Pi-Pi T shaped	1. LEU A:824, THR A:826, ASP A:793, SER A:796, SER A:828, TYR A:831, SER A:830, ALA A:800 2. LEU A:795 3. HIS A:827

Table II. Molecular docking analysis of *Syzygium aromaticum* extract (interactions)

Name of the Ligand	3D Interaction	2D Interaction
1,2-Bis (trimethylsilyl) benzene		
2,5-Cyclooctadien-1-ol		
2-tert-Butylphenol, trimethylsily ether		

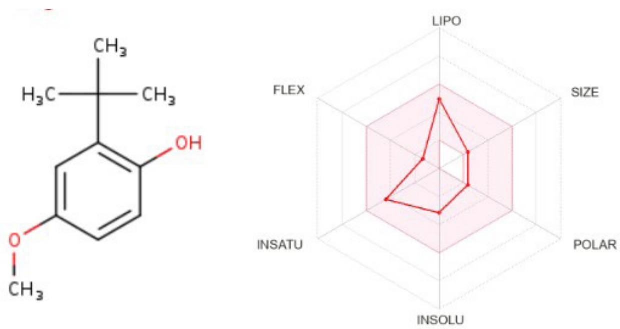


Figure 5: ADME analysis of the *Syzygium aromaticum* extract

at 3230. W: 2220 cm^-1, C=C stretching at 1588 cm^-1, Phenyl ring at 1602 cm^-1. 991 cm^-1, C-N stretching at 1342 cm^-1. wagging mode at 884 cm^-1, and C-O stretching at 1196. 944 cm^-1. These results align with other investigations that have described the existence of polyphenolic compounds and aromatic rings in clove, which are responsible for its biological effects and health benefits (20,21). A comparison of the mass spectra of the obtained peaks showed that there were several important peaks with retention times of 5. 610, 6. 015, 8. 544, 13. 200, 14. 843, 21. 060, and 23. 235 min, indicating the presence of more than one bioactive chemical. At the peak of 8, this action plan was found to be eugenol, which is found in clove oil, with antimicrobial and anti-inflammatory properties (22,23). This is in agreement with earlier studies in which eugenol was documented to be the main compound present in clove (24). At the peak 13, the first peak occurs at 78 min, close to the intensity of 200 min, which is corresponds to β-caryophyllene and the third peak occurs at 21. 60 min, pointing to humulene or other sesquiterpenes. These compounds are responsible for much clove's medicinal value by making it a useful analgesic and anti-inflammatory agent.

Consequently, phytochemical analysis revealed that clove contains a wealth of bioactive constituents with medical potential. The fibre content, which was confirmed by the presence of both flavonoids and terpenoids in the samples, suggests impressive antioxidant and anti-inflammatory properties, which are testified in the literature (20,25). Clove contains a large quantity of phenols and tannins, and therefore, it exhibits great astringent and antibacterial activity, which has been discussed and documented in various studies. The presence of fatty acids supports the heart and has anti-inflammatory properties, which, according to research, support the role of clove in being heart-healthy (26). There is a lack of alkaloids, saponins, glycosides, and proteins; hence, it has a unique phytochemical that might affect the therapeutic potential of clove. The DPPH assay of the plant bioactive components also revealed significant antioxidant activity of the clove. The percentage of inhibition increases as the concentration increases to 10μg/mL, which gave almost 75% inhibition which is with close proximity to the standard. The high antioxidant capacity of clove has been supported by many studies that have credited this attribute to its high phenol content, especially eugenol (27). The high inhibition rate observed at all concentrations clearly establish the high antioxidant capacity of clove, which is comparable to the industry's synthetic antioxidants (28).

The analysis of molecular docking showed that among the bioactive compounds from clove, 2-tert-Butylphenol, trimethylsily ether, and 1,2-Bis (trimethylsilyl) benzene present some interactions in two- and three-dimensional molecular spaces. These interactions imply that each of them provides the extract with specific traits related to its overall bioactivity. For example, 2,5-Cyclooctadien-1-ol could enhance bioassay and metabolic efficiency and 1,2-Bis(trimethylsilyl)benzene could help improve stability. Hence, the lipophilicity and membrane permeability of 2-tert-Butylphenol and trimethylsily ether are enhanced in developing drugs. These findings are consistent with other studies on the diverse treatment applications of clove compounds (29). Some important characteristics could be inferred from the ADME properties of eugenol, a bioactive compound derived from the clove. The molecular size of eugenol is moderate, and the compound is lipophilic; both aspects point toward favorable partition coefficient and suitable molecular weight. The extent of the polar surface area is not too high, which implies adequate solubility and absorption. However, in the case of low flexibility and less solubility reflected by a fixed conformation and limited extant solubility. The low unsaturation also increases stability because the resulting figure reveals a small possibility of double bonds. Hence, based on these ADME properties, one can infer that eugenol has a moderate to high clearance rate, which is optimal for medicinal purposes (30-32). Therefore, from FTIR, GC-MS, phytochemical tests, antioxidant activity tests, molecular dock analyses with selected standard drugs, and ADME studies on clove, it can be concluded that this plant contains various bioactive compounds, which can be useful in medical applications. These results are consistent with and justified by prior research on the biological activities and health benefits of clove. Clove contains polyphenolic compounds, aromatic rings, and many other bioactive chemicals; hence, it is considered the traditional use of clove in medicine.

CONCLUSION

This study validated the medicinal value of clove by examining its bioactive constituents and functional characteristics. Among all bioactive compounds, 2-tert-Butylphenol, trimethylsily ether has a high binding affinity on LEU A:795 protein. The notable antioxidant properties of the substance are attributed to the presence of essential functional groups and bioactive substances. Eugenol, a primary component of clove, has a pharmacokinetic profile that confirms its suitability for use in medical and pharmaceutical contexts. It exhibits excellent absorption, membrane permeability, and desirable pharmacological properties. These findings highlight the appropriateness of clove for use in therapeutic applications and its potential as a natural treatment.

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