INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Isolation, Evaluation, Purification and Antimicrobial Activity of Phytoconstituents from Clove

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ARTICLE INFO

Published: 16 Mar. 2025
Keywords:
Eugenol, Extraction, Purification, Evaluation.
DOI:
10.5281/zenodo.15034467

ABSTRACT

Clove (Syzygium aromaticum) (Family Myrtaceae) is one of the most important herbs in traditional medicine having a wide spectrum of biological activity. Phytoconstituents of clove comprise of various classes and groups of chemical compounds such as monoterpenes, sesquiterpenes, phenolic and hydrocarbon compounds. The major phytochemicals found in clove oil is mainly eugenol (70-85%) followed by eugenol acetate (15%) and β caryophyllene (5-12%). The present research work shows the antimicrobial activity of phytoconstituents of clove. Due to their antimicrobial activity. The current biological diagnostic findings in this literature review suggest that Clove are capable of being established as lead compounds.

INTRODUCTION

Spices and herbs have been used for thousands of centuries by many continents to enhance the flavour and aroma of foods; preserving foods and for their medicinal value. Syzygium aromaticum (Clove bud) is one of the most ancient and valuable spices of the Orient (Chaise et al., 2007) which are used as a carminative to increase hydrochloric acid in the stomach and to improve peristalsis. Essential oil compounds are fat soluble thus possess the ability to permeate the membranes of the skin before being captured by the microcirculation and drained into the systemic circulation which reaches all targets organs.

Chemical Constituents:

Various studies have been carried out to find various constituents of S. aromaticum [10-12]. Clove buds contain 15–20% essential oil, which is dominated by eugenol (70–85%), eugenol acetate (15%) and β-caryophyllene (5–12%). Other essential oil ingredients of clove oil are vanillin, crate Golic acid, tannins, Gallo tannic acid, methyl salicylate, flavonoids eugenin, kaempferol,

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

rhamnetin, eugenitin and triterpenoids like oleanolic acid.

Drug Design:

Drug Design is the innovative process of finding a new medication based on the knowledge of a biological target.

Collection of plant material:

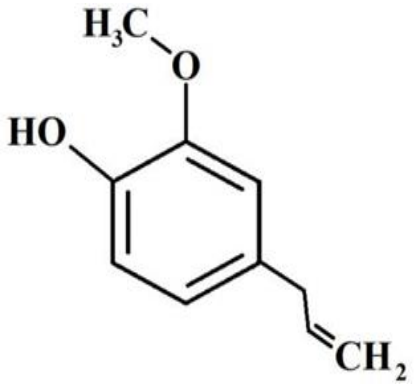
Around 250g of dried clove buds were purchased from the herbal store, Pune.

Introduction of Docking:

Three- dimensional structure is one of the foundations of structure-based drug/ligand design. often structural information of the protein and a drug/ligand are available separately but not together. Molecular docking is the process by which two molecules fit together in 3 D space. This can be achieved by probing the formation of intermolecular complexes using various computational methods. Generally, classical mechanics based force field methods are used in the molecular docking. Monte Carlo (MC) and Molecular Dynamics (MD) methods have also been employed to predict the best structural fit between protein and ligand molecules. The usefulness of Genetic Function Algorithm (GFA) is also illustrated in the literature. Most docking algorithms are able to generate a large number of possible. Structures and hence there is a need to score each structure to identify which are of most important. Thus docking problem is concerned with generation and evaluation of possible structures of protein-ligand complexes. The docking problem involves many degrees of freedom. There are six translational and rotational degrees of freedom of one molecule relative to other as well as the conformational degrees of freedom of each molecule. In the docking, by fixing the structure of receptor, the drug/ligands are moved to find the best 3D fit between the molecular surfaces. The receptor structure obtained from X-ray crystallography or NMR spectroscopy or molecular modelling building including homology methods. In the rigid body docking, since, the receptor cannot move, the degrees of freedom of the problem are those of the ligand: three translational, three global-rotational, and one internal dihedral rotation for each rotatable bond. Docking to a rigid receptor is thus an optimization problem over a 6 + n dimensional space, where is the number of rotatable bonds in the ligand. In the docking, the program obtains the image of the binding site from the molecular surface of the macromolecule and ligand molecules are mapped on to the binding and then docking energies and scores have been evaluated.

Preparation of Ligand:

A ligand structure was drawn on Marvin sketch application and saved in Mol2 format.



Preparation of Protein:

Open the PDB file of the protein i.e.7LID in Discovery Studio. When the file is open, press CTRL+ H. This shows the side panel containing the components of the PDB structure.

Preparation of Grid:

The protein file7LID_H.pdb was opened in Auto-Dock. The atoms ACH Cl Br I F SP HD N NAOA were added. In the “number of points in x, y and z dimensions”, slide to 60, 60 and 60 respectively. The grid file saves as output 7LID.gpf.

Preparation of Docking:

For the preparation of the docking parameter file (DPF), click on Docking > Macromolecule> Set rigid filament> Open in the ADT window to open the target PDBQT

Executing the commands for Docking:

Type the following command for running autogrid (Note: Change the protein name accordingly) 'autogrid4.exe–p3BQK.gpf– l 3BQK.glg'Wait until it shows 'complete' and then enter the following command for running Auto Dock. (Note: Change the ligand name accordingly) 'autodock4.exe–p amadiaquine. DPF –l amadiaquine. DLG' Wait until it shows complete.

Docking Analysis (RMSD Table)

Enter the Run which had minimum binding energy and maximum rank from the 'RMSDTABLE' i.e. Change RUN from'1' to a desired RUN value and press Enter. Check the Conformation Info window to see if ligand is interacting with the receptor.

Extraction:

Collection of Plant

Procedure: The dried clove bud samples were roughly crushed using a grinder. The samples were divided equally to produce 30g of grounded samples using different extraction methods.

Hydro Distillation:

The dried flower buds were used in obtaining the clove oil by steam distillation, using the Clevenger apparatus (Pyrex), as described by Harborne (1998). In brief, the dried flower buds were grinded and 30 g weighed out, placed in around bottom flask and water was added to approximately three-quarter full. The distillation apparatus was then connected to the flask. The trap arm was filled with water to allow the oil to condense on the water layer, heat was applied from the heating mantle, and as the water in the flask boiled, steam carrying the volatile oil rose through the neck of the flask condensing on the surface of the condenser onto the water on the graduated trap arm. Distillation was continued until there was no more difference in successive readings of the oil volume. The oil was drained off and dried over anhydrous sodium sulphate (BDH). The density of the oil was determined according to the weight: volume ratio (w/v)

Soxhlet:

The sample was placed in a Soxhlet extractor. Ethanol (90%) was placed in around flask along with the boiling chip granules. The extraction process continued for 6-7 hours at the temperature of 70-80 °C. The mixture was then filtered, and the solvent was evaporated using a rotary vacuum evaporator to obtain the crude extract. The extract was weighed and stored in a vial for further analysis.

Purification

TLC (Thin Layer Chromatography)

1.Thin layer chromatography (TLC) is mainly used qualitatively for screening different plant extracts, which serves as a very important tool in the overall phytochemical research studies.

2.Column Chromatography

Column chromatography is used for the separation and isolation of different constituents of extracts. Column chromatographic grade adsorbents of choice are used for packing the columns and various organic solvents are used as eluting solvents. first to plug the neck of the column with a wad of glass or cotton wool. Silica gel is either made into slurry with the solvent or as such introduced from the top of the column. The next step in column chromatography is to add the sample solution to the top of the column, in such a way that an arrow band is formed, for further elution. In order to fractionate the components of varying solubility, the gradient elution technique is followed. The column is subsequently eluted with various proportions of solvents in theorder of increasing polarity The fractions collected from the bottom are distilled to recover the solvent, while the concentrated residue of the fraction thus obtained, is stored in clean and dry glass vials and monitored by TLC studies.

Determination of antimicrobial activity:

To determine the antimicrobial activity, pour plate technique was performed. Based on the zone of inhibitions obtained by the extract, comparative

study of their qualitative antimicrobial activity was done. Test microorganisms taken for the study: Bacteria of soil were employed as a test organism. These includes.

Sterilization:

All the glassware used in the experiment was sterilized in hot air ovenat160C four hours. The media used in the experiments were sterilized in an autoclave at15 lbs per square inch for 15 minutes.

Composition of nutrient Agar Media:

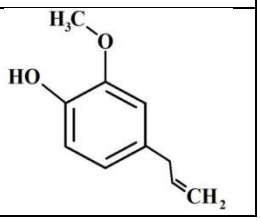
Nutrient agar medium was preferred as the media for bacteria. the following composition

Peptone: 0.5g Beef extract:0.5g Nacl:0.2g Agar:1g Distilled water:50ml

After preparation it was mixed well until the contents are dissolve and then autoclaved at 15lbs per square inch for 15 minutes Nutrient broth does not contain agar. Nutrient broth is used for enrichment of specified bacteria. Nutrient agar medium was prepared and poured in to sterilized petri dishes and placed it in laminar air flow.

RESULT:

RMSD Table:

Sr.NO	Binding Energy	Structure
Str-Eugenol	-6.95	

2DIMAGE:

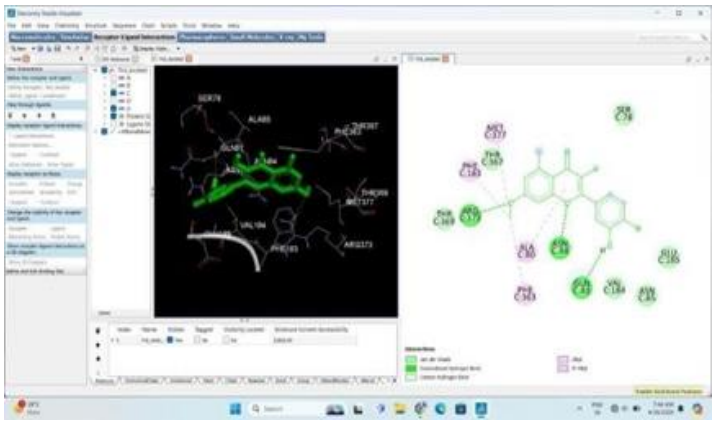


Fig.No. 1 2DIMAGE

Isolation and Purification:

Table No.2 Isolation and Purification

Sample	Time	Mobile Phase	Result
Extracted Sample	2 Min	Toluene: Ethyl Acetate 8:2	RF Value: 0.5

Biological Evaluation:

Examine all the culture for the presence of growth, which is indicated by turbidity in the Agarculture, appearance of yellow coloured growth on the slant surface and along the line of inoculation in the agars tab tube.

CONCLUSION:

A protein PDB 2w9h was selected from Rscbprotein Data Bank for binding the results of molecular docking revealed that the compounds bearing lowest binding shows more efficacy it also revealed that the designed molecules have good hydrogen bonding interactions with the important amino acids. Thus, docking study of the compounds were done amongst which compound/ ligands 6.95 showed binding energy than other compounds. The molecules were found to be interacted with protein 7lid. Thus, we can conclude that the synthesized compounds have a potential for further development as antimicrobial agent. The crude extracts were purified through column chromatography, which allowed for the isolation of individual bioactive compounds. This step was crucial for identifying and testing specific

compounds. The antimicrobial activities of the isolated compounds were tested against various pathogenic bacteria.

ACKNOWLEDGEMENT:

The authors wish to thank Dr. Sonali Banpure for her guidance's and valuable suggestion during the project. The authors acknowledge the support provided by Ms. Poornima Mam.

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HOW TO CITE: Sonali Bunpure*, Dhanashree Kherade, Pournima Bhalekar, Nita Tidke, Neelam Bhagdewani, Pramod Ingale, Saniya Hembade, Rutuja Pujari, Isolation, Evaluation, Purification and Antimicrobial Activity of Phytoconstituents from Clove, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 3, 1458-1462. https://doi.org/10.5281/zenodo.15034467