Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 3, https://carcinogenesis.com/

Essential Oils as Natural Inhibitors of Candida albicans Proteases: In-silico and In-vitro Evaluation

Meghana V Gowda[1], Netra B Hubballi[2], Sandeep V. Binorkar[3], Nivedita Patil[4], Dr. Rajendra Prasad M L[5], Nagabhushan V Charantimath[6], Anil kumar K M*[7], Jagadeep Chandra S*[8]

1Department of Biochemistry, Adichunchanagiri University (ACU), Karnataka, India

2Department of PG Studies in Kayachikitsa, JSS Ayurveda Medical College and Hospital, Mysuru-570028, Karnataka, India

3Department of Agadatantra, Government Ayurveda College, Vazirabad, Nanded-431601, Maharashtra, India

4Department of Biotechnology, M.S. Ramaiah University of Applied Sciences, Gnanagangothri Campus, New BEL Road, MSR Nagar, Bengaluru, Karnataka, India

5Department of Rasashastra and Bhaishajya kalpana, JSS Ayurveda Medical College and Hospital, Mysuru-570028, Karnataka, India

6Department of Zoology, Vijayanagar Sri Krishnadevaraya University, Ballari, Karnataka, India.

7Department of Environmental Science, JSS Academy of Higher Education & Research, Mysuru-570015, Karnataka, India.

8Department of Microbiology, JSS Academy of Higher Education and Research, Mysuru-570015, Karnataka, India.

Corresponding Author- jagadeepchandra@jssuni.edu.in , anilkumarenvi@jssuni.edu.in

ABSTRACT

Candida albicans is a common opportunistic fungal pathogen that causes mucosal and systemic infections, especially in immunocompromised persons. Hydrolytic enzymes including secreted aspartyl proteases (SAPs) and sterol 14α-demethylase (CYP51) contribute to the pathogen's pathogenicity by facilitating tissue invasion, biofilm development, and immune evasion. In this study, chosen essential oils lemongrass, thyme, and tea tree were investigated for their inhibitory ability against SAP5 and CYP51 using in-silico docking and in-vitro antifungal tests. Molecular docking indicated that substances such as thymol, geraniol, citral, and citronellol had high binding affinities and hydrogen bond interactions with critical SAP5 and CYP51 active site residues. Subsequent enzyme inhibition assays and microbiological casein agar plate tests proved that lemongrass and thyme oils had strong antifungal action, whereas tea tree oil had comparatively weaker effects. These findings imply that essential oils, notably lemongrass and thyme, have potential as natural antifungal agents by targeting important C. albicans proteases. Further research into identified active chemicals may lead to new therapy options for Candida infections that are resistant.

How to Cite: Meghana V Gowda, Netra B Hubballi, Sandeep V. Binorkar, Nivedita Patil, Dr. Rajendra Prasad M L, Nagabhushan V Charantimath, Anil kumar K M, Jagadeep Chandra S, (2025) Essential Oils as Natural Inhibitors of Candida albicans Proteases: In-silico and In-vitro Evaluation, *Journal of Carcinogenesis*, *Vol.24, No.3s*, 719-737.

INTRODUCTION

Since ancient times, essential oils have been used in many different traditional healing methods all over the world, because of their biological functions.The word 'aromatherapy' has now become eminent in promoting health and well being using plants natural extract. Essential oils have gained clear importance in biomedicine because they can destroy a variety of bacterial, fungal, and viral pathogens(Sharifi-Rad et al., 2017). Many studies documented several properties of essential oil, including antioxidant, anti-cancer, anti-inflammatory, anti-fungal and antimicrobial properties in variety of cell and animal models*(Mohamed Abdoul-Latif et al., 2023).*

Essential oils are synthesized naturally from different plant parts during the process of secondary metabolism, they are

complex volatile compounds. These oils contain the characteristic volatile compounds responsible for the plant's aroma. Each essential oil is differentiated with its unique aromatic compound. More than 250 different types of essential oils are known (origanum oil, tea tree oil, clove oil, *menthe piperita* oil, lemongrass oil, thyme oil, sassafras oil pippermint oil, ginger grass oil etc are some of the well known essential oil)*(Swamy et al., 2016).*

A Survey of literature reveals that some of the essential oils listed below have antifungal activity and are effective against opportunistic fungi such as candida species. Candida spp. are most common cause of fungal infection in human and can increase the mortality rate upto 40% in immunocompromised patients. *Candida albicans* is the most common species responsible for candidiasis *(Abd Rashed et al., 2021).*

C.albicans is a part of the human microbiota that colonises the skin, mouth, and gastrointestinal tract of healthy individuals asymptomatically. These fungal species can cause superficial dermal and mucosal infections like vaginitis, thrush, and esophagitis in both immunocompetent and immunocompromised individuals, including patients undergoing chemotherapy or those with AIDS. A significant virulence feature of *C. albicans* is its ability to form biofilm *(Talapko et al., 2021).* The infection caused by *C. albicans* is commonly known as candidiasis. Candidiasis can be classified into two categories depending upon the severity of the disease. The first category includes mucosal infections, among which thrush is the best known, characterized by white patches in the infected membranes. These infections generally affect gastrointestinal epithelium , vaginal mucosa, or oropharyngeal region. Furthermore, Vulvovaginal Candidiasis (VVC) is quite common among women, and some of them experiences repeated occurrences of this infection, which is known as Recurrent Vulvovaginal Candidiasis (RVVC) *(Talapko et al., 2021) (Tamo, 2020).*

The hydrolytic enzyme secreted by microorganism are the major factors in pathogenesis. *C. albicans* produces several hydrolytic enzymes, including secreted aspartyl proteinase, lipases, and phospholipase B and Sterol 14**α**-demethylase (CYP51), which is required for ergosterol biosynthesis in fungi(Schaller et al., 2005). Secreted aspartyl protease(SAP) has been identified as one of the virulent factors responsible for different types of candidiasis. It is an extracellular proteolytic enzyme secreted by *candida albicans*. SAPs allow for hyphae formation, adhesion, and phenotypic switching, as well as the digestion of host cell membranes and the evasion of host immune defenses by weakening and inactivating the human central nervous system and complement system(Naglik et al., 2003). SAP is comprised of ten enzymes, varying from SAP1 to SAP10. Each of these enzymes has the ability to cause virulence. compared to other enzymes in SAP family, SAP5 is liable to cause more virulence. The SAP enzyme digests molecules in order to obtain nutrients, disrupts the host cell membrane for invasion and tissue damage, and attacks the host cell immune system in order to shield itself from antimicrobial attacks by the organism or host(Schaller et al., 2005).

Sterol 14**α**-demethylase (CYP51) is an ancestral member of the cytochrome P450 superfamily that is necessary for fungi to synthesis ergosterol(Erg11). CYP51 is the primary target of clinical azole drugs used to treat fungal infections. CYP51 is a member of the CYP superfamily. According to a recent analysis CYP51 (ERG11) can also have a variety of indirect roles *(Becher & Wirsel, 2012)*. The deletion of CYP51 in *C.albicans* reduces mycelial elongation and invasive development and causes defects in the elimination of reactive oxygen species, resulting in reduction of virulence. Ergosterol serves a special physiological function, but it is also commonly used in the manufacture of pharmaceuticals *(Zhang et al., 2019).* The majority of antifungal medications in clinical use are designed to inhibit the main enzymes involved in ergosterol biosynthesis. Almost 30 enzymes known as Erg proteins are involved in the complex de novo ergosterol biosynthesis. Mevalonate biosynthesis, farnesyl pyrophosphate biosynthesis, and ergosterol biosynthesis are the three modules that make up the ergosterol biosynthesis pathway *(Ahmadipour et al., 2021).*

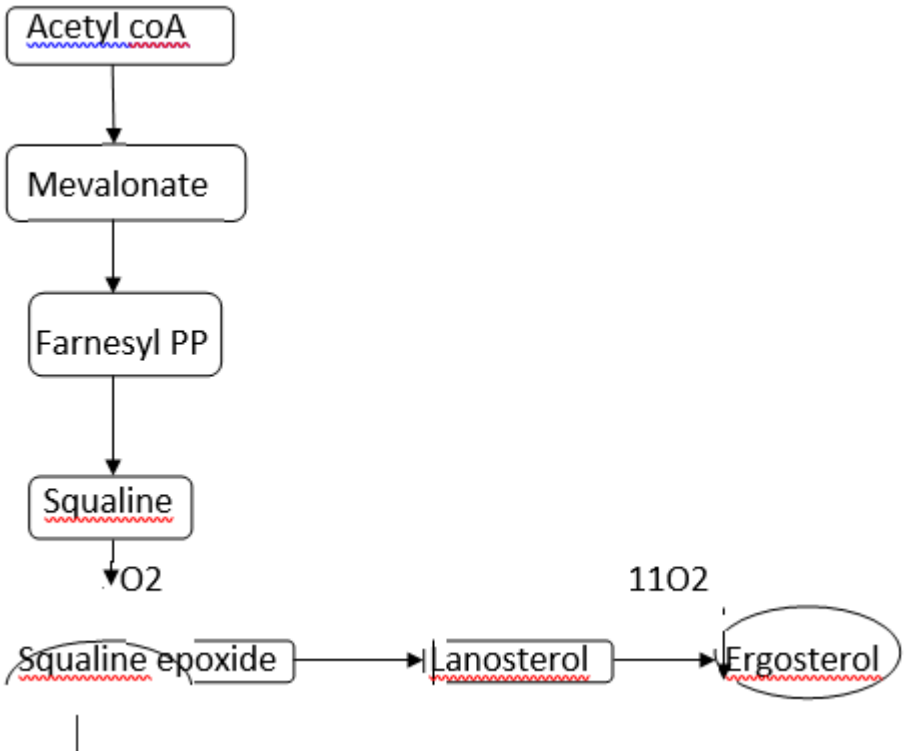


Figure 1: Ergosterol synthesis pathway

Table 1: List of essential oils, compounds and their plant source.

Sl.no	Plant source	Essential oils	Compounds
1.	Origanum vulgare	Origanum	Carvacrol, p-cymene, α-terpineol, borneole
2.	Melaleuca alternifolia	Tea tree oil	1,8 cineole, terpinen 4-ol, α-terpineol, γ-terpinene, α-pinene, p-cymene
3.	Syzygium aromaticum	Clove oil	Eugenol, caryophyllene, vanillin
4.	Cinnamomum zeylanicum	Cinnamon oil	Cinnamaldehyde, eugenol, cinnamic aldehyde
5.	Ginger grass	Ginger grass oil	Zingerone, zingiberene, citral, cineol
6.	Santalum album	Sandalwood oil	Santol, santene, sesquiterpene, santalene
7.	Rosmarinus officinalis	Rosemary oil	Boreneol, cineole, camphene, α-pinene, camphor

8.	Mentha piperita	Peppermint oil	Menthol, menthone, limonene, sesquiterpine, apigenin, menthofuran, methyl acetate
9.	Eucalyptus globulus	Eucalyptus oil	Cineole, caryophyllene, limone, eucalyptol
10	Sassafras albidum	Sassafras oil	Safrol, camphor, geranyl acetate
11.	Cymbopogon citratus	Lemon grass oil	Geraniol, myrcene, neral citral, citronellol, luteolin
12.	Thymus vulgaris	Thyme oil	p-cymene, carvacrol, thymol
13.	Cymbopogon	Citronella oil	Piperitone , elemol
14.	Ocimum basilicum	Basil oil	Linalool, methyl chavicol(estragole), methyl eugenol
15.	Citrus sinensis	Orange peel oil	Octanol, decanal, dodecanal, neral

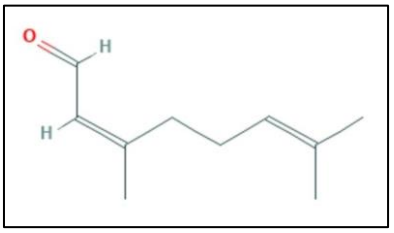
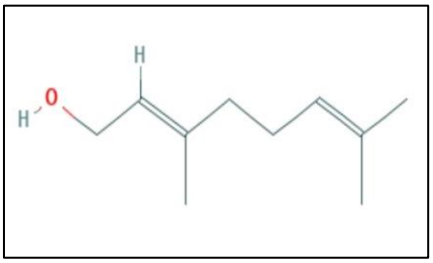
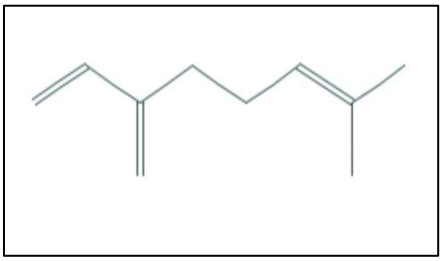
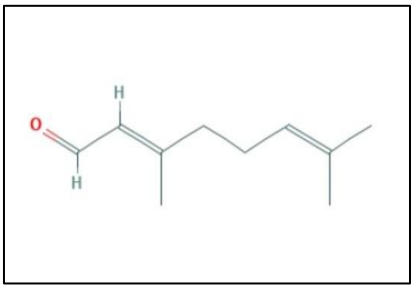
MATERIALS AND METHODS

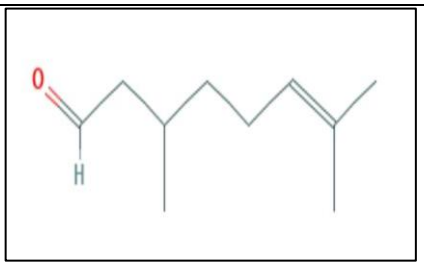
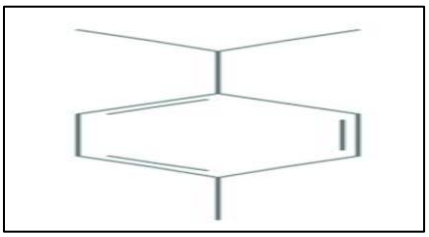
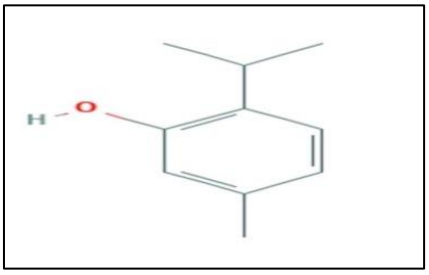
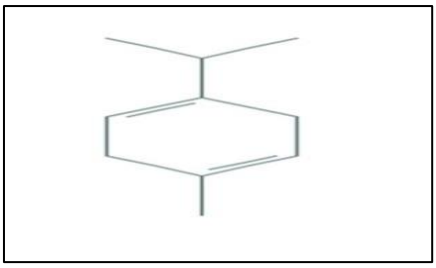
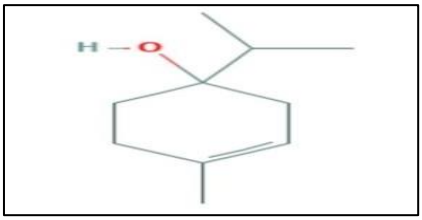
Compounds from three major essential oils were used in this study. Lemon grass oil contains Neral(31.5%), Geraniol(31.53%), Myrcene(16.61%), Citral(26.1%), Citronellol(3.83%). Thyme oil contains p-cymene(18.41%),Thymol(47.59%), g-Terpinene(30.90%). Tea tree oil conatins Terpinen-4-ol(41.5%), g-Terpinene(21.2%), Alpha terpineole(10.2%), 1,8 cineole(2.1%)(Mukarram et al., 2021).

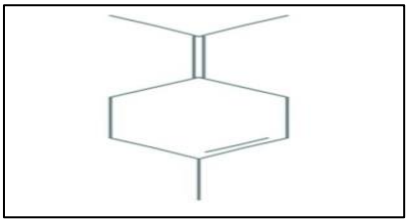
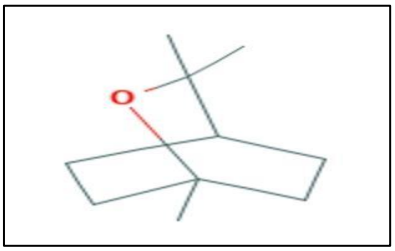
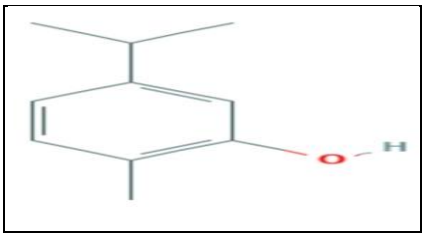
Tea tree oil [TTO] is extracted from the leaves of the Melaleuca alternifolia shrub, which grows in New South Wales of Australia. It is an essential oil comprising about 100 components, the majority of which are monoterpenes, sesquiterpenes, and related alcohols. **Lemon grass oil [LGO]** was extracted from the lemon grass plant that grows in tropical and subtropical parts of the world. It may be pale or bright yellow in colour, with a thin consistency and with lemony scent(Carson et al., 2006). **Thyme oil [THO]** is extracted from fresh leaves and flowers of the thyme plant. Thymol was the most widely recognized component, followed by γ-terpinene(Escobar et al., 2020).

These three essential oil have shown antifungal activity cited in pubmed literature(Nazzaro et al., 2017), thus these oils and their compounds were chosen to investigate how they interact with SAP5 and CYP51 enzymes active site of *candida albicans*. The below-mentioned compounds structures, molecular formulas, and chemical formulas were obtained from the PubChem database and tabulated in Table 2.

Table 2 : Essential oil compounds with IUPAC names, Molecular formula, and 2D structures

Sl.No	Compounds	IUPAC Name	Molecular Formula	Compound's 2D structure
1.	Neral	(2Z)-3,7-dimethloctadiene-2,6-dien-ol	$C_{10}H_{18}O$	
2.	Geraniol	(2E)-3,7-dimethyloctate-2,6-diene-1-ol	$C_{10}H_{18}O$	
3.	Myrcene	7-methyl-3-methylideneocta-1,6-diene	$C_{10}H_{16}$	
4.	Citral/geranial	(2E)-3,7-dimethylocta-2,6-dienal	$C_{10}H_{16}O$	
5.	Citronellol	3,7-dimethyloct-6-en-1-ol	$C_{10}H_{20}O$	

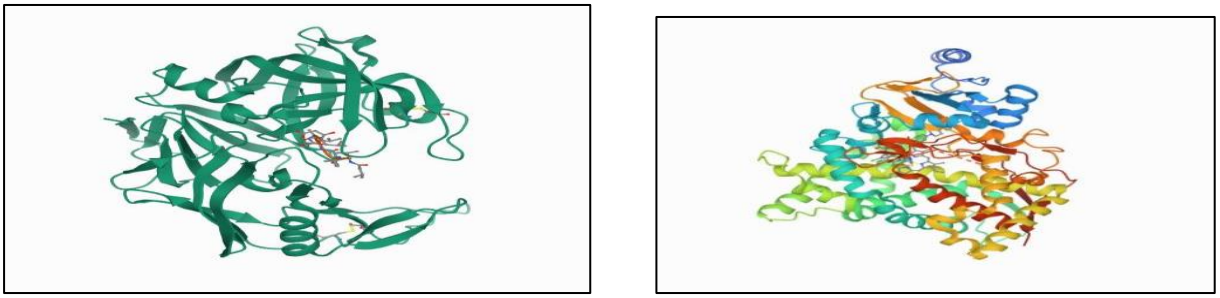
				
6.	p-cymene	1-methyl-4-propan-2-ylbenzene	$C_{10}H_{14}$	
7.	Thymol	5-methyl-2-propan-2-ylpropanol	$C_{10}H_{14}O$	
8.	γ-terpinene	1-methyl-4-propan-ylcyclohexa-1,4-diene	$C_{10}H_{16}$	
9.	Terpine 4-ol	4-methyl-1-propan-2-ylcycloheax-3-en-1-ol	$C_{10}H_{18}O$	

10.	Terpinoline	1-methyl-4-propan-2ylidenecyclohexene	$C_{10}H_{18}O$	
11.	1-8 Cineol	4,6,6-trideuterio-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane	$C_{10}H_{18}O$	
12.	carvacrol	2-methyl-5-propan-2-ylphenol	$C_{10}H_{14}O$	

(a). Protein structures

The three-dimensional structure of proteins SAP5 and CYP51 was generated using the protein data bank (PDB). The PDB is a well-known open-access online database that informs users with the Structure, properties, and functions of a wide range of macromolecules like nucleic acids. The obtained protein structures are shown below in **Figure 2(A) and 2(B).**

The PDB ID of SAP5 and CYP51 proteins are 2QZX and 5V5Z, respectively. SAP5 is a ten-membered family of acidic hydrolases, with activity ranging from pH 3.0 to 7.0. This protein is composed of two chains, A and B. Each chain consists of 342 amino acid residues. whereas CYP51 contains only one chain consisting of a 537 amino acid sequence.



(a) **(b)**

Figure 2: (a) Secreted aspartyl protease (SAP5) (PDB ID:2QZX) (b) Lanosterol 14α-dimethylase(CYP51) (PDB ID:5V5Z).

To visualize and edit or modify the downloaded proteins, the software pyMol was used. water molecules and residues of amino acids that are not standard, found linked to the protein, were also removed in this software. It was further cleaned with Argus Lab. Another in-silico tool, Castp, was used to recognize the ligand-binding site pocket region of the chosen proteins before commencing the docking studies.

(b). Protein structure validation

Using Galaxy Web, a protein refining tool, the protein structures were obtained. After refinement, the generated protein structures were confirmed and assessed using the Procheck Ramachandran plot from RAMPAGE for dihedral angles (ψ) versus ϕ of residues of amino acid and verified favourable residue for docking experiments. 92% and above is a highly favourable condition of protein, and it is known as a stable molecule for docking studies.

(c). Docking studies and visualization

Docking studies and molecule interaction were conducted to understand the interaction between the selected compounds and proteins using PyRx v0.8 (PyRx v0.8 is an open-access software with Virtual Screening). The protein and ligands that were previously selected, loaded in PyRx in PDB format, as well as the active binding site residues retrieved using Castp, were protected with a Grid box, and the grid box was adjusted for the chosen binding site residues to acquire the highest orientation with the lowest binding affinity values (Kcal/mol).

The docking results obtained from PyRx were visualized using the softwares, BIOVIA Discovery Studio Visualizer and LIGPLOT+ v1.4.4. These softwares were used to visualise the bond length, Hydrophobic interaction, and Hydrogen bonds among the proteins and decided compounds. BIOVIA Discovery Studio Visualizer help in 3-Dimensional visualization, while the Ligplot+vl.4.4. helps to generate 2-Dimensional interaction among respective proteins and ligands. Among all the docked compounds, compounds showing the lowest corresponding binding affinity were selected is listed below in the **table 3 and 4.**

Invitro anti-fungal assay

(a). Caseinolytic protease assay

Proteases are enzymes that break down peptide bonds**.** The activity of proteases may be determined using as a standardised procedure called a protease activity test(protease assay). Here, casein plays the role of substrate.

Materials:

Casein, Tris-Hydrochloric acid (tris-HCl) as buffer, sodium carbonate (Na2Co3) , Trichloroacetic acid (TCA) sodium carbonate (Na_2CO_3), Folin–Ciocalteu reagent(FC reagent).

Casein 0.4ml ,Tris-Hcl Buffer with PH 8.5 was prepared and incubated with various essential oil concentrations as inhibitors and 10µl and 20µl pancreatic enzyme at 37ºC separately for 2.5 hours (pancreatic enzyme was used as the standard enzyme in this antifungal assay). Later, 1.5 ml of 0.44M trichloroacetic acid(TCA) was added to cease the reaction, which was prepared earlier and allowed to stand for 30 minutes. Simultaneously, non-inhibitor control trials were conducted. The mixture was centrifuged at 1500 rpm for 15 min. The aliquot of 1ml supernatant was collected in a separate test tube. The inhibitors and enzyme were added, again to balance the mixture, and 2.5ml of sodium carbonate added followed by 0.5ml of F-C reagent. The colour developed was read at 660nm using UV-Spectrophotometer.

Anti-fungal Assay

(a).Casein agar plate assay.

Potato dextrose agar (PDA) was prepared along with 1% (w/v) casein and poured into petridishes. The plates were allowed to solidify for 30 minutes (all the petri dishes and PDA were autoclaved to sterilize before using). The discs dipped with *candida albicans* culture were placed, among three wells, one well was considered as a control without inhibitor, the other two wells were poured with essential oils with different concentration(10µl, 20µl, 30µl, and 40µl). plates were kept in an incubator at 37ºC for 24 hours. After 24hours incubation, adding TCA stopped the reaction and inhibition can be clearly visualized by the decrease in the diameter of the zone of *candida albicans* wells with the inhibitor when compared to that of the control plate figure10, which comprised of *candida albicans*.

RESULT AND DISCUSSION

In in-silico analysis, before conducting a molecular docking, the protein validation was carried out to ensure that the protein was suitable and stable for docking studies. The selected proteins, after refinement with Castp, showed more than 90%. i.e., protein showed SAP5 96.76% and CYP51 showed 91% (Table5) which is now eligible and stable for further studies .

Table 3: Values shown by Ramachandran plot from RAMPAGE of favourable, allowed & outlier regions.

Sl.no	Protein structure	Residues in favourable region(%)	Residues in allowed region (%)	Residues in outlier region (%)
1.	2QZX	96.76	1.49	0
2.	5V5Z	91.0	7.8	0.7

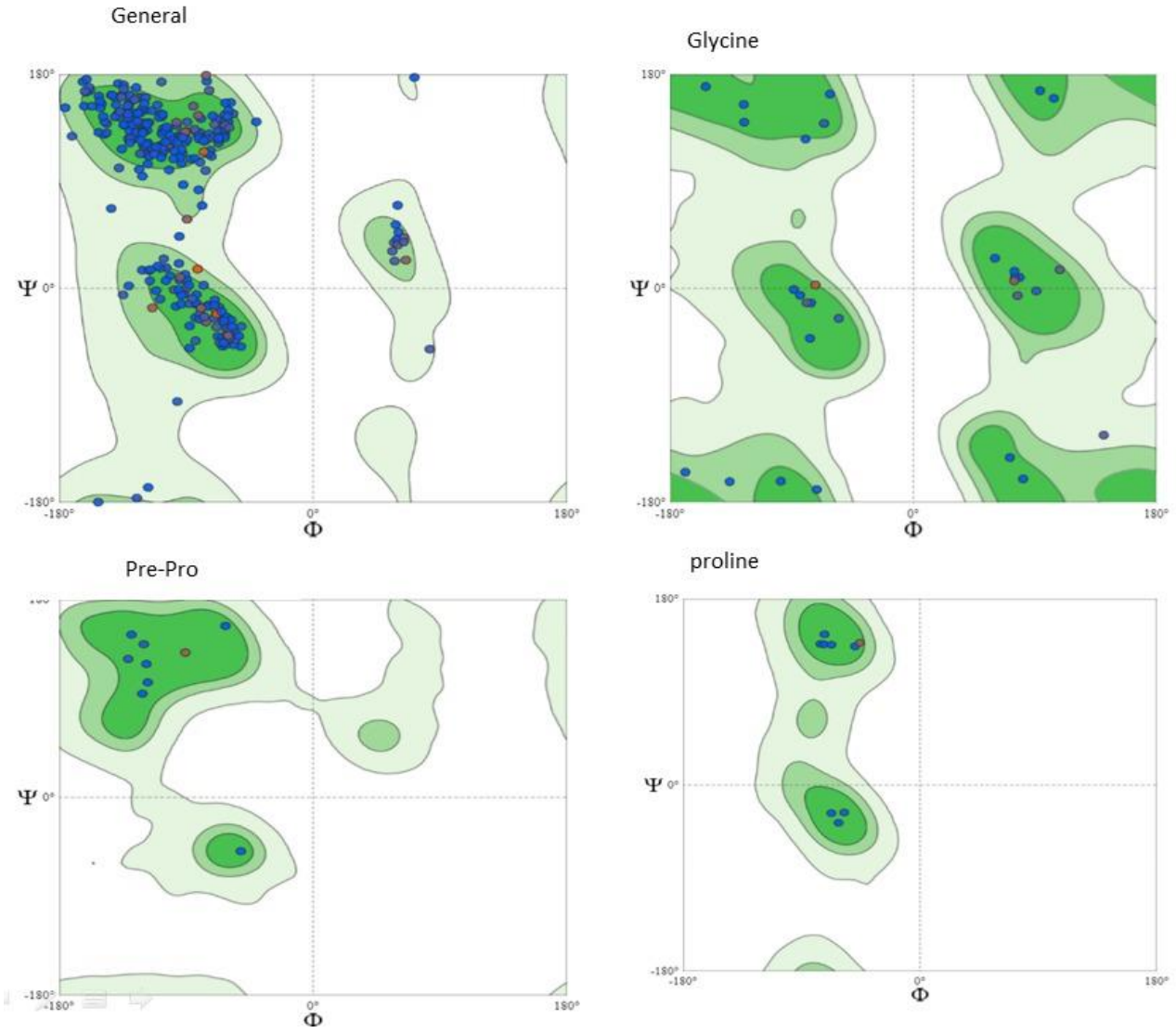


Figure 3A: SAP5(2QZX) protein's Ramachandran Plot generated by RAMPAGE exhibiting verified region for ψ (backbone dihedral angles) against φ (Amino Acid residues).

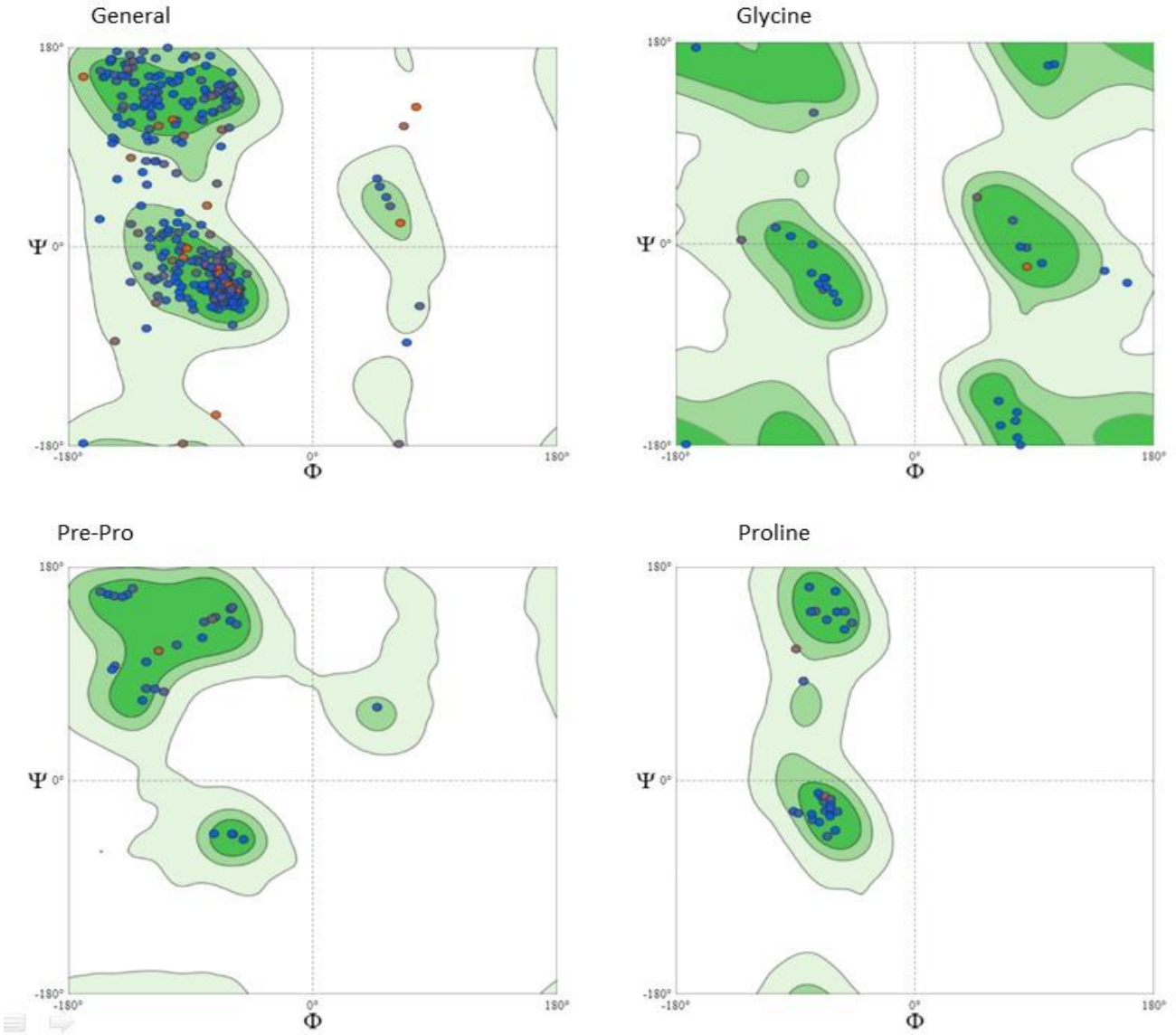


Figure 3B: CYP51(5V5Z) protein's Ramachandran Plot generated by RAMPAGE exhibiting verified region for backbone dihedral angles ψ against Amino Acid residues φ

(a). SAP5 (2QZX) protein interaction with specific ligands in Molecular Docking

Table 3 shows the molecular docking and visualisation of protein SAP5(2QZX) with essential oil constituents, their binding affinity, and amino acid residues.

Among all these compounds, thymol is a main component of thyme oil, showed the highest binding affinity that is -5.9Kcal/mol, and total number of interactions (polar/non polar) were 10. This is followed by alpha- terpineole and terpinen-4-ol having binding affinity - 5.7Kcal/mol and total number of interactions, 7 and 9, respectively. Binding affinity and amino acid interactions with SAP5 protein can be clearly understood in Table 3. But as we consider the hydrogen bonding interaction to clearly depict the docking results, we considered compounds showing with highest hydrogen bonds after visualisation. Geraniol, citronellol, and citral, which exhibited more hydrogen bonds, are considered in analyzing the docking results(Figure 6)

Table 4: Results of molecular Docking of essential oil compounds with SAP5 (PDB ID: 2QZX) protein.

Sl.no	Compound CID	Compound	Binding affinity (Kcal/mol)	Binding of amino acid Residues
1.	643779	Neral	-5.3	Tyr84, Ile123, Asp32, Gly220, Ser88
2.	638011	citral	-5.4	Ile30, Ile123, Gly220, Tyr84, Asp32
3.	442501	Alpha tepineole	-5.7	Gly220, Ile123, Ser88, Asp32, Tyr84, Gly85

4.	637566	Geraniol	-5.3	Arg120, Ile30, Tyr84, Asp32, Asp86, Ile123
5.	2758	1,8 cineole	-5.6	Ile30, Ile123, Asp86, Tyr84, Gly220, Ser88
6.	7794	citronellol	-5.3	Tyr84, Thr13, Ile12, Thr222, Ile30, Gly220, Asp32, Ile123
7.	7463	P cymene	-5.5	Arg120, Ala119, Asp86, Ile123, ser88, ile30, Gly220, Asp32, Tyr84
8.	7461	Gamma Terpinene	-5.5	Arg120, Ala119, Asp86, Ser88, Tyr84, Asp32, Gly220, Ile30, Ile123
9.	11230	Terpine 4-ol	-5.7	Tyr84, Asp32, Ile12, Arg120, Ile30, Thr13, Gly220, Ile123
10.	31253	Myrcene	-5.1	Trp51, Arg120, Ala119, Asp86, Tyr84, Ser88, Asp32, Gly220, Ile123, Ile30
11.	6989	Thymol	-5.9	Asp32, Ile123, Tyr84, Asp86, Ile30, Gly220, Arg120, Ile12, Thr13
12.	10364	Carvacrol	-5.9	Lys257, Lue280

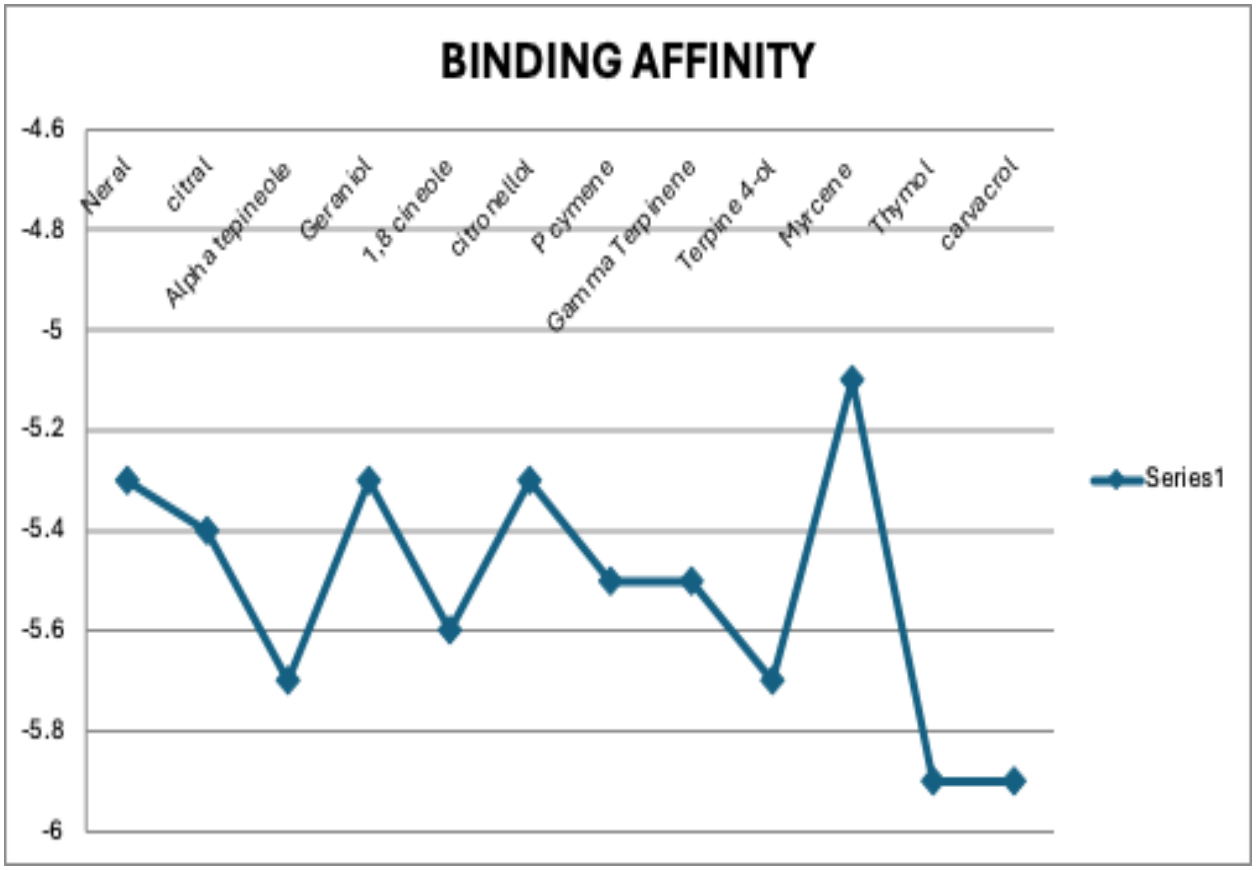


Figure 4: Line graph showing molecular docking results between compounds of Essential oil with SAP5 (PDB ID: 2QZX)

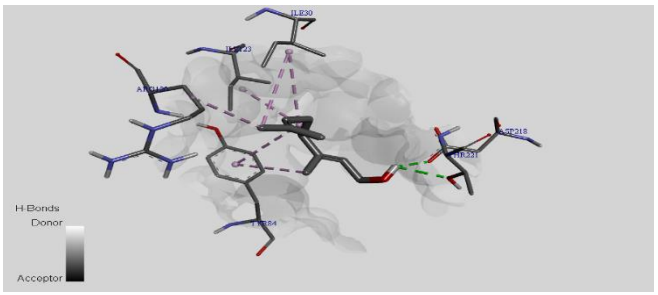
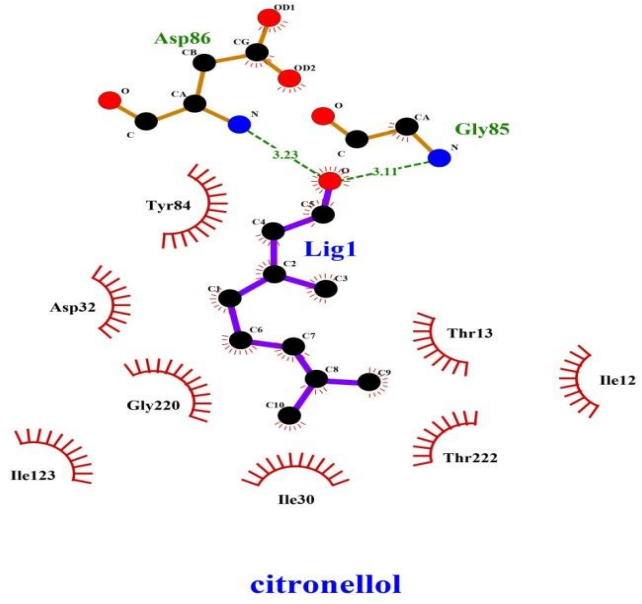


Figure 5a: 2-D and 3-D images showing residual interactions of the compound Geraniol with active site region of protein with 2QZX (SAP5).



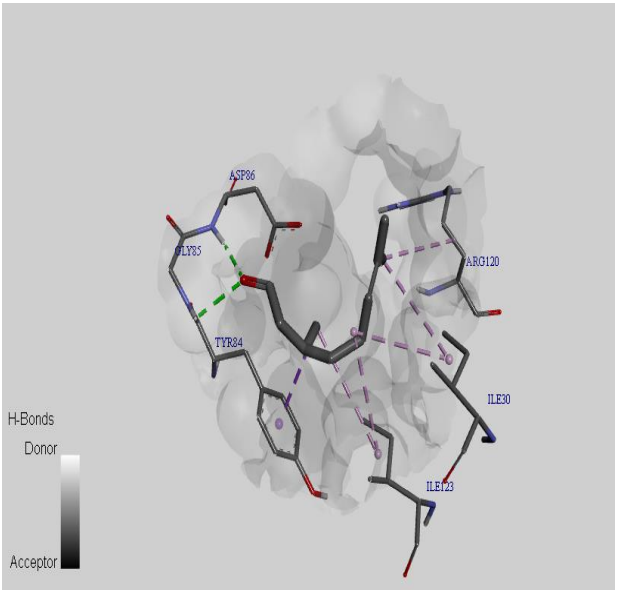
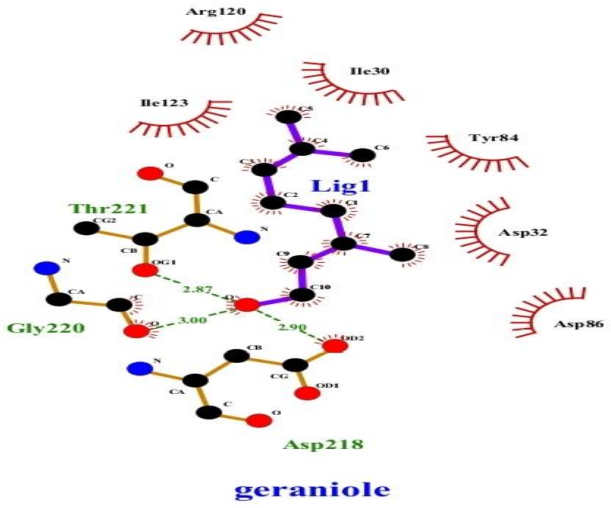


Figure 5b: 2-D and 3-D residual interactions of the compound Citronellol wactive site region of protein wit 2QZX (SAP5).

Citronellol



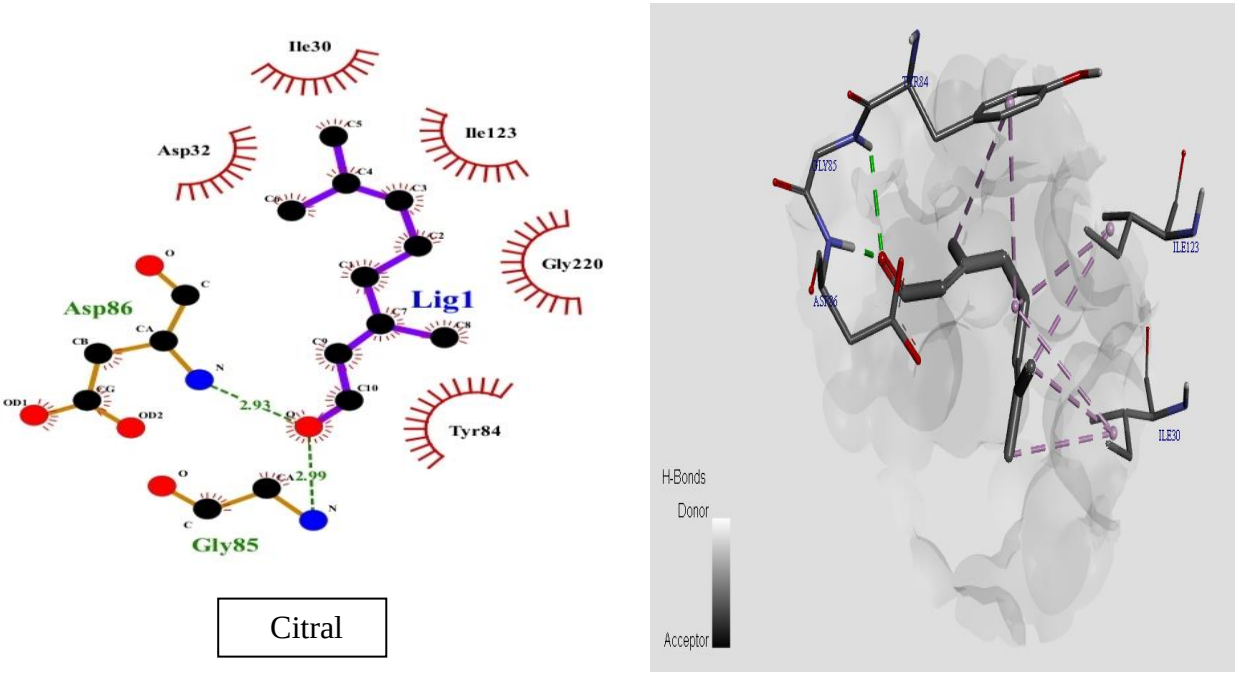


Figure 5c: **2-D and 3-D images showing residual interactions of the compound Citral with active site region of protein with 2QZX (SAP5).**

(b): CYP51 (5VZV) protein interaction with specific ligands in Molecular Docking

Table 4 shows the molecular docking and visualisation of protein CYP51(5V5Z) with essential oil constituents, their binding affinity, and amino acid residues.

Table 5: Molecular Docking results of Essential oil compounds against CYP51 (PDB ID:5V5Z) protein

Sl.no.	Compound CID	Compounds	Binding Affinity	Binding Residues of amino acids
1.	643779	Neral	-6.8	Leu121, Leu376, Phe233, Met508, Leu121, Tyr118
2.	638011	citral	-6.1	His373, Phe380, Pro230, phe233, leu87, Lys90
3.	442501	Alpha tepineole	-6.2	Phe223, Leu376, Met508, Phe380, Leu121, Tyr118, Tyr132, Thr122
4.	637566	Geraniol	-5.8	Met508, Tyr118, Leu376, phe380, Tyr132, Thr122, Phe228, Leu121, Phe233
5.	2758	1,8 cineole	-5.7	Phe380, His377, Ser507, Met508, Phe233, Leu376, Ser378
6.	7794	citronellol	-5.9	Leu121, Leu376, Met508, Tyr118, Thr122, Phe283
7.	7463	P cymene	-6.1	Ser378, Met508, Leu121, Phe233, Tyr118, Phe380, Leu376, Thr122, Tyr132
8.	7461	Gamma Terpinene	-6.3	Met508, Ser378, Phe380, Leu376, Phe233, Leu121, Tyr118, Tyr132, Thr122
9.	11230	Terpine 4-ol	-6.2	Met508, Phe380, Leu376, Ser378, Phe233, Tyr118, Leu121, Tyr132, Thr122
10.	31253	Myrcene	-10.1	Pro230, Met508, Phe233, Phe380, Leu87, Lys90, Tyr64
11.	6989	Thymol	-6.2	Leu121, Leu376, Tyr118, Phe380, Phe233, His377, Ser378
12.	10364	carvacrol	-6	Leu376, Leu121, Tyr118, Phe233, Phe380, Ser378,

				His377

Molecular Docking evaluation and Visualisation of CYP51(5V5Z) can be clearly interpreted by the table. Out of all the components docked, myrcene shows the highest binding affinity of 10.1kcal/mol followed by neral and gamma terpinene showed binding affinity of -6.8 Kcal/mol, and -6.3 Kcal/mol, respectively. Next to these compounds alpha terpineole and terpine 4-ol shows a binding affinity of -6.2 Kcal/mol. Binding affinity, interaction, and amino acid residues of protein and ligands can be clearly interpreted through Table 4. But as we mainly focus on the number of hydrogen bonds formed after visualization as a docking study, Geraniol, citronellol, and Neral were considered for analyzing the docking results.

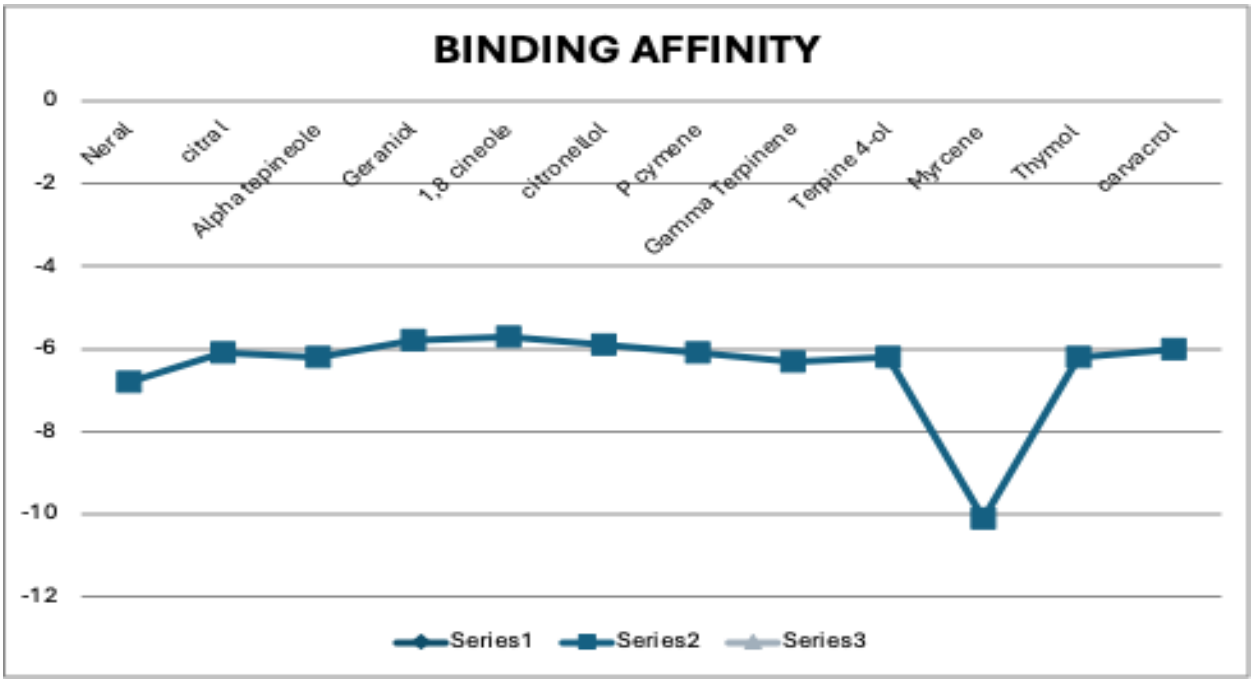


Figure 6: Line graph showing molecular docking results between compounds Essential oil with CYP51 protein (PDB ID: 5V5Z) (x-axis – compounds , y-axis – binding affinity)

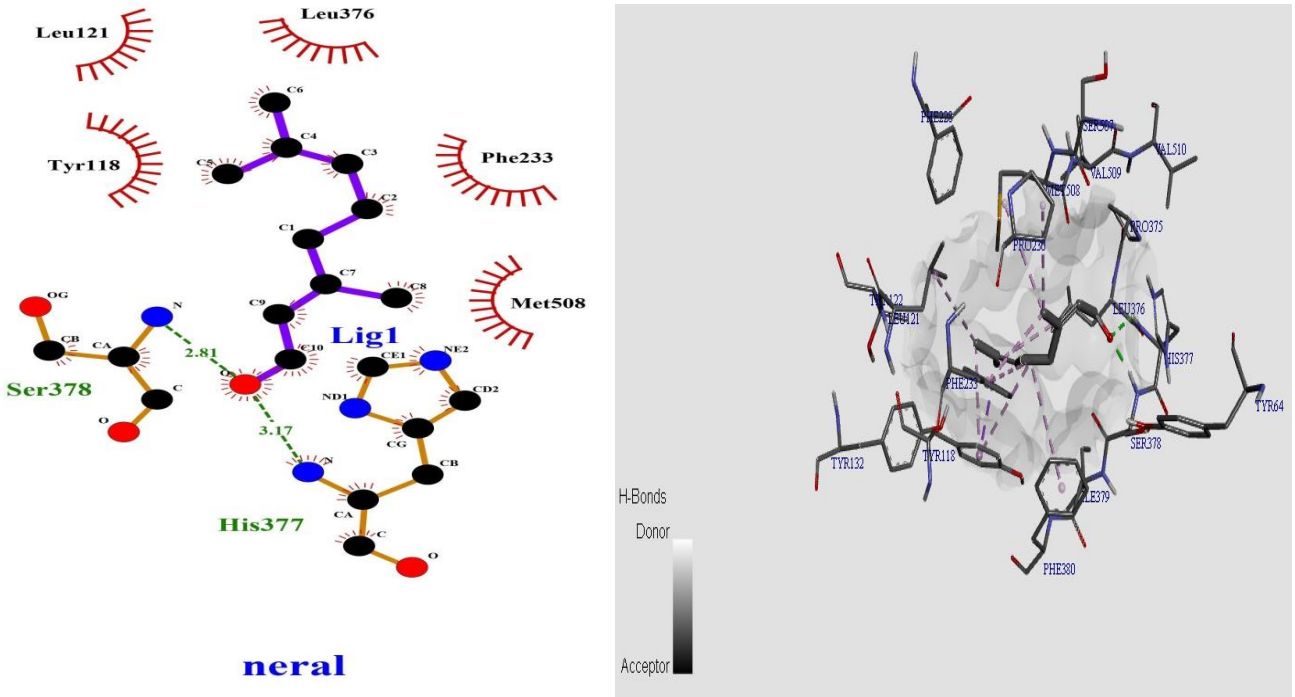


Figure 7a: 2-D and 3-D images showing residual interactions of the compound Neral with active site region of protein with 5V5Z (CYP51).

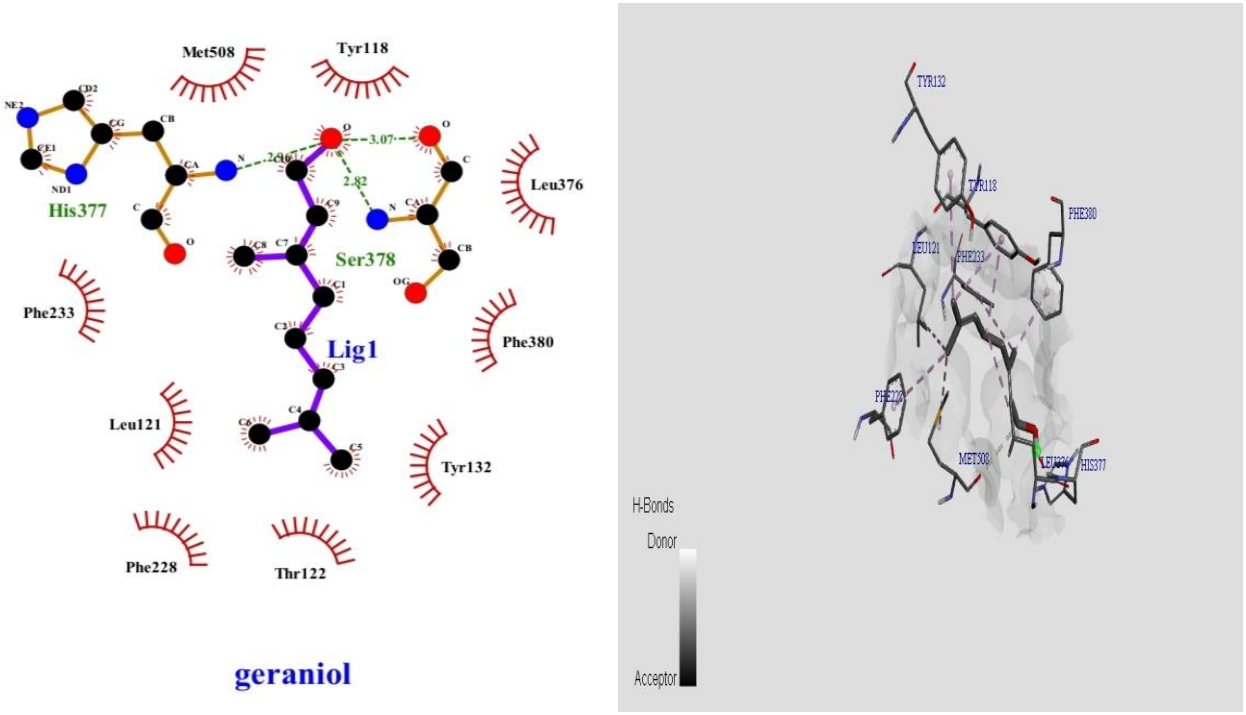


Figure 7b: 2-D and 3-D images showing residual interactions of the compound Geraniol with active site region of protein with 5V5Z(CYP51).

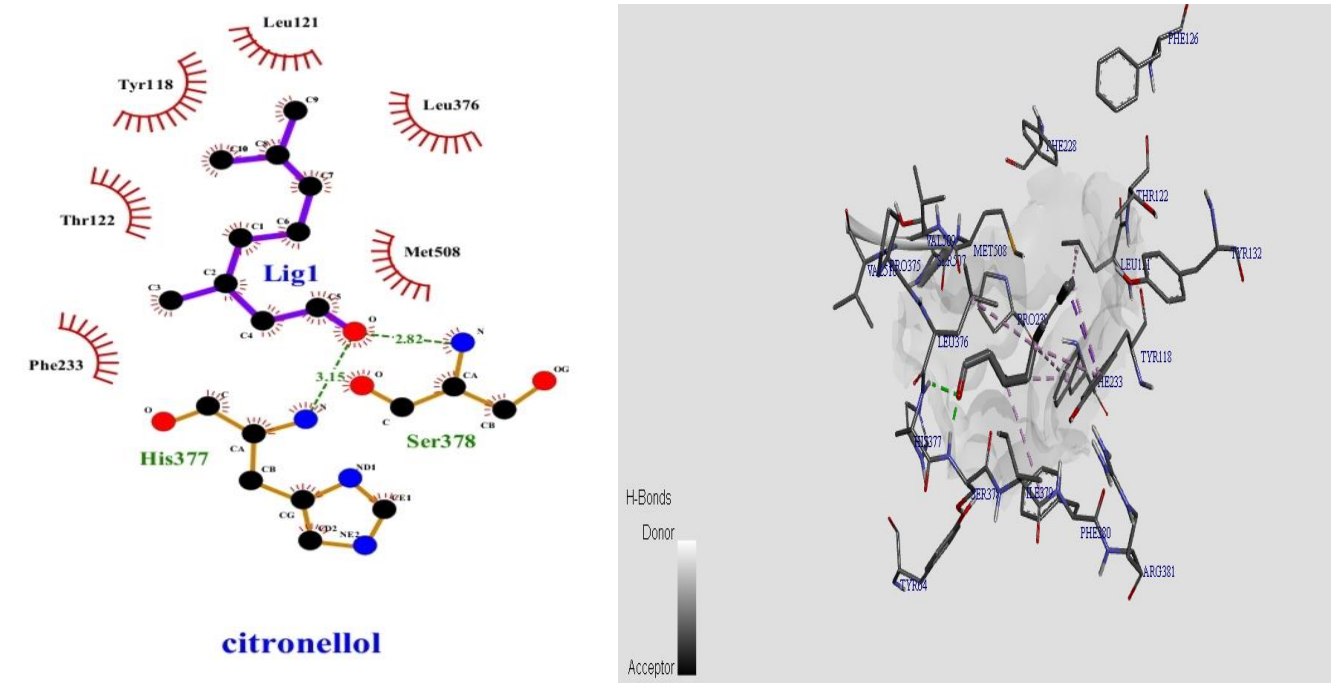


Figure 7b: 2-D and 3-D images showing residual interactions of the compound Citronellol with active site region of protein witl 5V5Z(CYP51

Table 6: Molecular docking analysis of selected compounds with SAP(2QZX), CYP51(5V5Z) enzymes of *candida albicans*.

Sl.no	proteins	compounds	Binding affinity	Number of Hydrogen bonds	Hydrogen bonds
1.	2QZX	Geraniol	5.3	3	Thr221, Gly220, Asp218
		citronellol	5.3	2	Asp86, Gly85
		citral	5.4	2	Gly85, Asp86
2.	5V5Z	Geraniol	6.1	2	Ser378, His377
		Neral	6.8	2	His377, Ser378
		citronellol	5.9	2	His377, Ser378

From the above docking results, Nerol, Geraniol,Citral and Citronellol have shown maximum hydrogen bonds compared to other compounds. The obtained results of protein shows maximum hydrogen bonds with three major compounds, like SAP(2QZX)binds with compounds of essential oil and shows maximum hydrogen bonds with citral, geraniol, and citronellol. Whereas CYP51(5V5Z) shows maximum hydrogen bonds with geraniol, citral, citronellol. Geraniol interacts with 2QZX(SAP5) and 3 hydrogen bonds and binds to the active site region Thr221, Gly220, Asp218 whereas citronellol and citral shows 2 hydrogen bonds and binds to the same active site regions of Asp86 and Gly85. Geraniol, neral, citronellol interact with 5V5Z(CYP51) and show 2 hydrogen bonds, and all three binds to the same active site region of His377, Ser378(Table 6). As a result, the obtained results can be used to guide future research into the use of these compounds to treat *candida albicans*. Thereby, based on insilico studies, lemongrass oil and thyme oil can be use to inhibit SAP5 and CYP51 enzymes of *Candida albicans*.

***In-vitro* antifungal assay**

Protease assay with casein as substrate with standard enzyme, pancreatic enzyme was used to investigate protein activity. *Candid albicans* live culture and *Candida albicans* supernatant were replaced with pancreatic enzyme later. Thyme oil exhibited increase inhibition than Lemon Grass oil

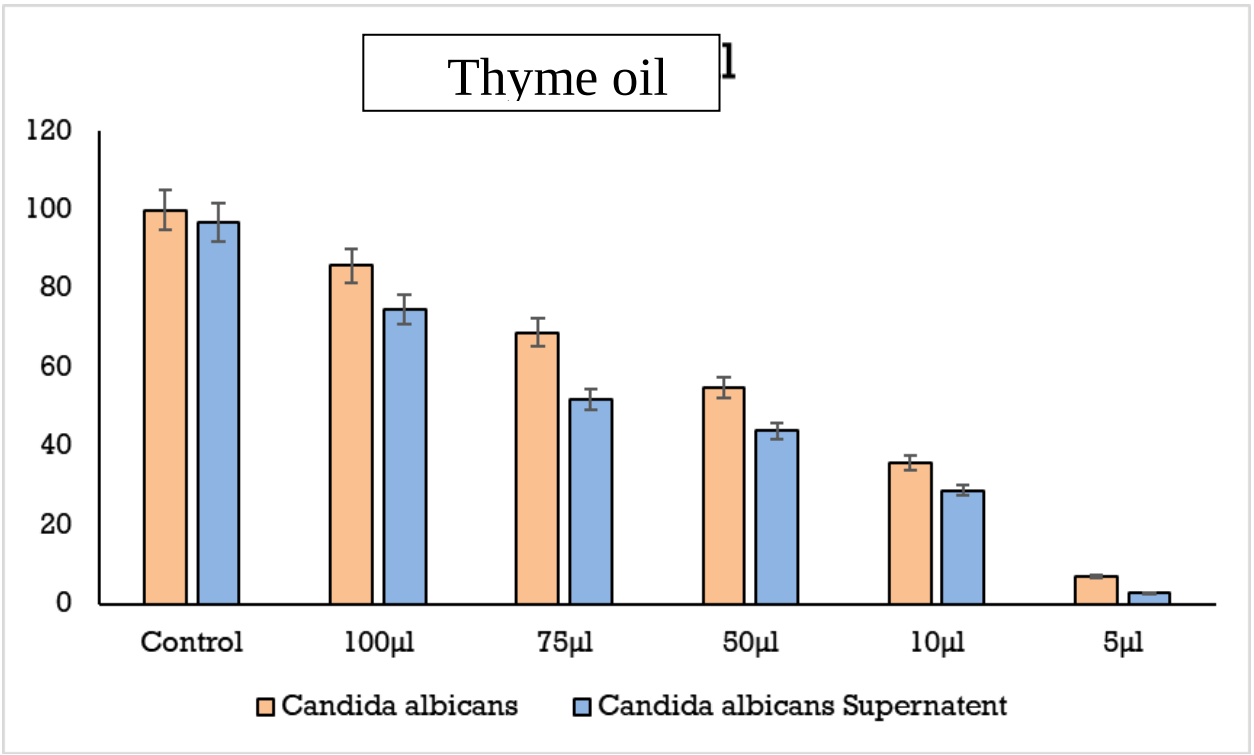


Figure 8a: Histogram representing activity of *Candida albicans* live culture and Candida albicans supernatant with Thyme oil as a inhibitor(x-axis- concentration of thyme oil, y-axis- percent of inhibition).

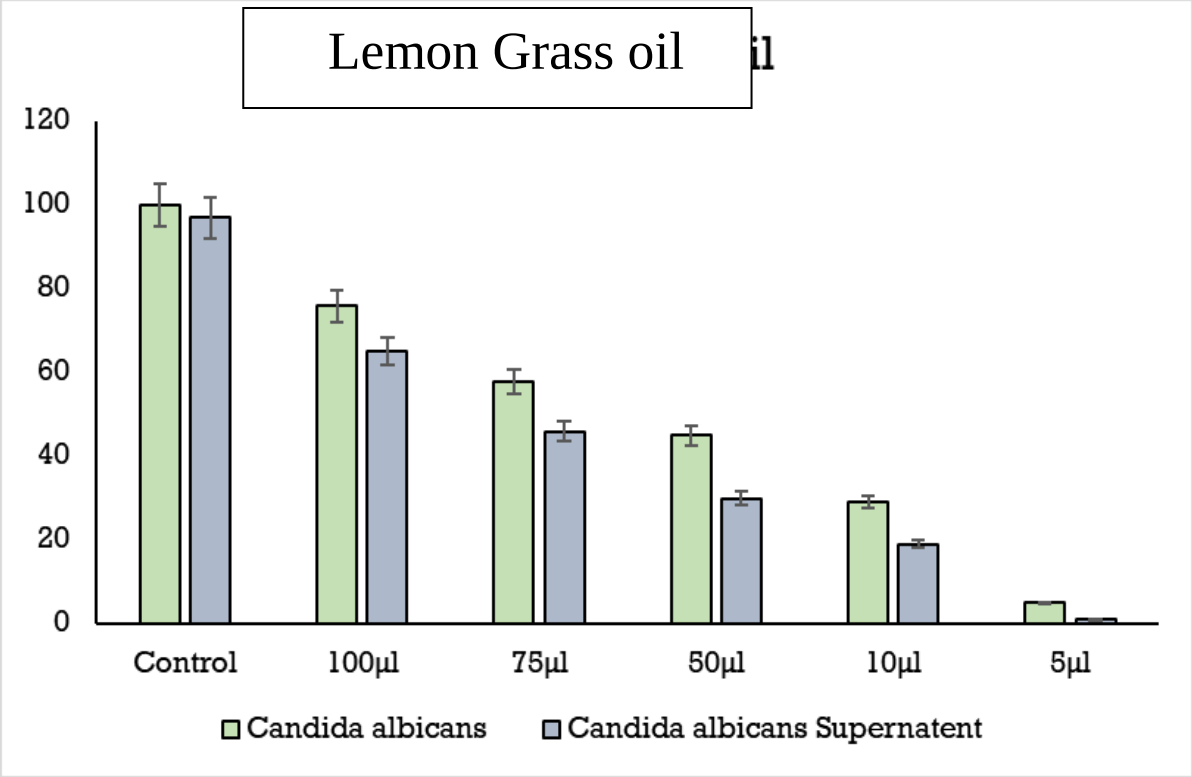


Figure 8b: Histogram representing activity of *Candida albicans* live culture and *Candida albicans* supernatant with Lemon Grass oil as an inhibitor(x-axis- concentration of Lemon Grass oil, y-axis percent of inhibition).

Caseinolytic plates assay was further carried out to provide strong evidence for antifungal activity of essential oils. It is a visual method for detecting Protease Inhibitors. Casein agar plates were prepared ideally, and the disc diffusion method was carried out to visualize the inhibitory effect of essential oils on *Candida albicans*. The wells with a higher concentration of oil show more inhibitory effects. To conclude this microbial assay results, Casein agar plate assay should be done in triplets.

DISCUSSION AND CONCLUSION

Natural antifungal products are a fascinating new therapeutic replacement to synthetic drugs. Essential oils stand out among natural plant products due to their widespread use in standard healing systems around the world. Our studies, along with previous studies, give strong evidence that essential oils and their compounds act effectively as antifungal agents, and show great inhibitory effects of fungi. In the present study insilico analysis where three oils were major components were selected, and which compounds present in Lemon Grass oil and Thyme oil showed more inhibitory activity compared to compounds of Tea Tree oil. Based on these results, Invitro studies (enzyme assay) were carries out. Additionally, for stronger evidence, Microbial assay (casein agar plate assay) was conducted. The result obtained supported the insilico work results.

In summary, we can conclude that Lemon Grass oil and Thyme oil exhibited maximum inhibitory effects, whereas Tea Tree oil shows minimum effect on the enzymes of *Candida albicans*. On the whole, we wind up with the conclusion that essential oils shows a clear interst in antifungal activites. Further research can be conducted by extracting particular proteins from *candida albicans* live cultures and performing invitro work to effectively demonstrate the inhibitory effect of Lemon Grass oil and Thyme oil on *Candida albicans*.

REFERENCES

[1] Abd Rashed, A., Rathi, D.-N. G., Ahmad Nasir, N. A. H., & Abd Rahman, A. Z. (2021). Antifungal Properties of Essential Oils and Their Compounds for Application in Skin Fungal Infections: Conventional and Nonconventional Approaches. *Molecules*, *26*(4), 1093. https://doi.org/10.3390/molecules26041093

[2] Ahmadipour, S., Field, R. A., & Miller, G. J. (2021). Prospects for anti-Candida therapy through targeting the cell wall: A mini-review. *The Cell Surface*, *7*, 100063. https://doi.org/10.1016/j.tcsw.2021.100063

[3] Becher, R., & Wirsel, S. G. R. (2012). Fungal cytochrome P450 sterol 14α-demethylase (CYP51) and azole resistance in plant and human pathogens. *Applied Microbiology and Biotechnology*, *95*(4), 825–840. https://doi.org/10.1007/s00253-012-4195-9

[4] Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). *Melaleuca alternifolia* (Tea Tree) Oil: A Review of Antimicrobial and Other Medicinal Properties. *Clinical Microbiology Reviews*, *19*(1), 50–62. https://doi.org/10.1128/CMR.19.1.50-62.2006

[5] Escobar, A., Pérez, M., Romanelli, G., & Blustein, G. (2020). Thymol bioactivity: A review focusing on practical applications. *Arabian Journal of Chemistry*, *13*(12), 9243–9269. https://doi.org/10.1016/j.arabjc.2020.11.009

[6] Mohamed Abdoul-Latif, F., Ainane, A., Houmed Aboubaker, I., Mohamed, J., & Ainane, T. (2023). Exploring the Potent Anticancer Activity of Essential Oils and Their Bioactive Compounds: Mechanisms and Prospects for Future Cancer Therapy. *Pharmaceuticals*, *16*(8), 1086. https://doi.org/10.3390/ph16081086

[7] Mukarram, M., Choudhary, S., Khan, M. A., Poltronieri, P., Khan, M. M. A., Ali, J., Kurjak, D., & Shahid, M. (2021). Lemongrass Essential Oil Components with Antimicrobial and Anticancer Activities. *Antioxidants*, *11*(1), 20. https://doi.org/10.3390/antiox11010020

[8] Naglik, J. R., Challacombe, S. J., & Hube, B. (2003). *Candida albicans* Secreted Aspartyl Proteinases in Virulence and Pathogenesis. *Microbiology and Molecular Biology Reviews*, *67*(3), 400–428. https://doi.org/10.1128/MMBR.67.3.400-428.2003

[9] Nazzaro, F., Fratianni, F., Coppola, R., & Feo, V. D. (2017). Essential Oils and Antifungal Activity. *Pharmaceuticals*, *10*(4), 86. https://doi.org/10.3390/ph10040086

[10] Schaller, M., Borelli, C., Korting, H. C., & Hube, B. (2005). Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses*, *48*(6), 365–377. https://doi.org/10.1111/j.1439-0507.2005.01165.x

[11] Sharifi-Rad, J., Sureda, A., Tenore, G., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M., Ademiluyi, A., Sharifi-Rad, R., Ayatollahi, S., & Iriti, M. (2017). Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. *Molecules*, *22*(1), 70. https://doi.org/10.3390/molecules22010070

[12] Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evidence-Based Complementary and Alternative Medicine*, *2016*(1), 3012462. https://doi.org/10.1155/2016/3012462

[13] Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., & Škrlec, I. (2021). Candida albicans—The Virulence Factors and Clinical Manifestations of Infection. *Journal of Fungi*, *7*(2), 79. https://doi.org/10.3390/jof7020079

[14] Tamo, S. P. B. (2020). Candida Infections: Clinical Features, Diagnosis and Treatment. *Infectious Diseases and Clinical Microbiology*, *2*(2), 91–102. https://doi.org/10.36519/idcm.2020.0006

[15] Zhang, J., Li, L., Lv, Q., Yan, L., Wang, Y., & Jiang, Y. (2019). The Fungal CYP51s: Their Functions, Structures, Related Drug Resistance, and Inhibitors. *Frontiers in Microbiology*, *10*, 691. https://doi.org/10.3389/fmicb.2019.00691

REFERENCES

[1] WHO Immunization Data portal - Detail Page. Immunization Data. 2023. URL: https://immunizationdata.who.int/global/wiise-detail-page/human-papillomavirus-%28hpv%29-vaccination-coverage

[2] Padma TV. India resolves to reduce cervical cancer by vaccinating girls. www.gavi.org. 2024. URL: https://www.gavi.org/vaccineswork/india-resolves-reduce-cervical-cancer-vaccinating-girls

[3] Mehra R, Ray A, Kumari A, Kaur A, Hora R, Quadri SF, et al. Google Trends for the Human Papillomavirus Vaccine in India From 2010 to 2024: Infodemiological Study. J Med Internet Res. 2025 May 27;27:e69729. DOI: 10.2196/69729.

[4] Devnath, R., & Sharma, K. (2024). Government's bold initiatives: tackling cervical cancer in India with determination and commitment. *Current Medical Research and Opinion*, *40*(9), 1647–1649. DOI: 10.1080/03007995.2024.2388221

[5] Pal D, Sahoo BK, Taywade M, Maji S. Evidence of Knowledge, Attitude, and Practice Regarding Human Papilloma Virus Vaccination at the Community Level in India: A Systematic Review and Meta-analysis. Asian Pac J Cancer Prev. 2024 Mar 1;25(3):793-800. DOI: 10.31557/APJCP.2024.25.3.793

[6] Shah P, Shetty V, Ganesh M, Shetty AK. Challenges to Human Papillomavirus Vaccine Acceptability among Women in South India: An Exploratory Study. Am J Trop Med Hyg. 2021 Aug 9;105(4):966-973. DOI: 10.4269/ajtmh.20-1650

[7] Union Minister Dr Jitendra Singh Advocates for United Efforts to Tackle Cervical Cancer at "Together Against HPV" Conclave. Pib.gov.in. 2017. URL: https://www.pib.gov.in/PressReleseDetail.aspx?PRID=2085299&utm_

[8] Swarnapriya K, Kavitha D, Reddy GM. Knowledge, Attitude and Practices Regarding HPV Vaccination Among Medical and Para Medical in Students, India a Cross Sectional Study. Asian Pac J Cancer Prev. 2015;16(18):8473-7. DOI: 10.7314/apjcp.2015.16.18.8473

[9] Chowdhury S, Ara R, Roy S, Tanvir SMS, Eva FN, Neela TM, Moonmoon AA, Sifat S, Zamila M, Hawlader MDH. Knowledge, attitude, and practices regarding human papillomavirus and its' vaccination among the young medical professionals and students of Bangladesh. Clin Exp Vaccine Res. 2022 Jan;11(1):63-71. DOI: 10.7774/cevr.2022.11.1.63

[10] Krokidi E, Rao AP, Ambrosino E, Thomas PPM. The impact of health education interventions on HPV vaccination uptake, awareness, and acceptance among people under 30 years old in India: a literature review with systematic search. Front Reprod Health. 2023 May 5;5:1151179. DOI: 10.3389/frph.2023.1151179

[11] Kataria I, Siddiqui M, Treiman K, Foley S, Anand M, Biswas S, et al. Awareness, perceptions, and choices of physicians pertaining to human papillomavirus (HPV) vaccination in India: A formative research study. Vaccine X. 2022 Oct 15;12:100228. DOI: 10.1016/j.jvacx.2022.100228

[12] Noreen K, Naeem Khalid S, Murad MA, Baig M, Khan SA. Uptake and determinants of HPV vaccination in South Asia: a systematic review and meta-analysis. Front Public Health. 2024 Dec 11;12:1453704. DOI: 10.3389/fpubh.2024.1453704