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Phytochemical Constituents in Essential Oil of *Cymbopogon Citratus* and Exploring the Potential of Antifungal Activity for Management of Phytopathogenic Fungi

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Abstract

Essential oil was extracted from the *Cymbopogon citratus* (Lemongrass) plant to evaluate its antifungal activity against four phytopathogenic fungi, viz., *Fusarium oxysporum*, *Alternaria alternata, Aspergillus flavus,* and *Aspergillus niger,* using the poisoned food technique. Different concentration of extracted essential oil viz., 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm, and 6000 ppm, were used to evaluate the potential effect against tested pathogens. At the concentration of 1000 ppm, 100% inhibition of mycelial growth was observed against *Alternaria alternata* and *Aspergillus niger,* whereas at a concentration of 2000 ppm 100% growth inhibition was found against *Fusarium oxysporum* and *Aspergillus flavus* fungi, respectively. The percentage inhibition of mycelial growth of the tested fungi was found to be dose dependent. The GC-MS analysis of essential oil revealed 40 compounds. Among them 2,6-Octadienal, 3,7-dimethyl- (E)- (Citral), Neral (Beta Citral), 2-Pentanone, 4-hydroxy-4-methyl- (Diacetone alcohol, Geraniol (Lemanol) and Geranyl acetate were found to be major constituents. *In-silico* studies revealed that Geranyl acetate has strong binding affinity and inhibition constant against four major fungal cell wall proteins. Thus, based on the above results it concluded that essential oil of *Cymbopogon citratus* could be used as an alternative to synthetic fungicides for management of phytopathogenic fungal diseases.

Keywords: Antifungal Activity; Lemongrass; Essential Oil; Phytopathogenic Fungi; Molecular Docking; GC-MS

Introduction

Phytopathogens such as fungi, bacteria, nematodes, and viruses cause numerous diseases in plants. Among these phytopathogens, fungi are the major causal agent of numerous diseases lead to the loss of production of many economically important crops [1]. Annually 10–15% losses of major crops worldwide are associated with plant diseases, which cause direct economic losses of hundreds of billions of dollars[2]. About 70–80% of the plant diseases are mainly caused by phytopathogenic fungi, and they adversely affect crop growth and yield [3]. In the recent agricultural system, infection of crops by fungal phytopathogens is increasing seriously day by day, and they have adversely affected crop production, crop quality, and are becoming a bottleneck towards sustainable agricultural developments [4]. The excess use of synthetic fungicides reported as non-biodegradable adds residual toxicity, leading to environmental pollution. Hence, in recent times, plant-derived secondary metabolites have gained more attention for disease and pest management as plant metabolites are eco-friendly.

Among the plant metabolites, essential oils are mixtures of organic and volatile substances, mainly composed of terpenoids and phenylpropanoids with a generally molecular weight of > 300 Da [5]. Among the vast numbers of phytochemicals present in the oil, terpenoids represented a large group of phytochemicals with potent antimicrobial activity [6]. The potent activity or action of the essential oil mainly depends on the combined effect of both active and inactive constituents. The inactive compounds of the essential oil may influence the rate of reaction, reabsorption, and bioavailability of the active compounds. Essential oils and their derivatizing compounds are gaining more attention because of their safe use, wide acceptance by users, and potent multipurpose functional use. More than 17,500 species of angiospermic plants produced essential oils [7]. Among them, the essential oil of Cymbopogon species (Lemongrass) encompasses a number of bioactive compounds. The use of this essential oil has a very long history, as it has been used as a traditional medicine since very ancient times. Post-harvest management of fungal disease by using essential oils has also been well documented [8]. The mechanism of the antifungal action of essential oils is still not well understood because of their complex nature [9].

Considering the rapid increase in the incidence of fungal infection and the development of drug-resistant strains, there is an urgent need for novel drugs with less toxic effects and greater effectiveness. Essential oil possesses the antifungal properties to act as an inhibitor of cell wall-associated enzymes; hence, it offers a key therapeutic agent to treat fungal infections. The fungal cell wall is a rigid mechanical barrier mainly composed of structural components like chitin, glycosal phosphatidyl inositol anchors (GPI), glucan, and mannoproteins that protect the fungus against various environmental stresses as well as other osmotic forces [10]. Hence, these fungal cell wall-based components open an excellent target site for antifungal drug design. For fungal cell wall construction, the enzymes chitin synthetase, UDP-glycosyltransferase, and glucosamine-6-phosphate synthetase play an important role. The enzyme chitin synthase plays an excellent role in the chitin biosynthesis pathway [11], whereas UDP-glycosyltransferase is involved in the first step of glycosyl phosphatidyl inositol GPI biosynthesis [12] and glucosamine-6-phosphate synthetase takes part in the synthesis of N-acetylglucosamine, regarded as the essential building blocks for the cell wall chitin [13]. Another protein, Sterol 14 α-Demethylase Cytochrome P450 is involved in ergosterol biosynthesis; hence, this protein gains attention for molecular targets [14]. In the present study essential oil was extracted from *Cymbopogon citratus* plant by hydro-distillation methods through Clevenger's apparatus. *In-vitro* antifungal studies was conducted to evaluate the antifungal potential of essential oil against four phytopathogenic fungi such as *Alternaria alternata*, *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum.* GC-MS analysis was performed to investigate the chemical composition profile of essential oil. An *in-silico* studies was carried out by taking the four important chemical constituents of the essential oil against four fungal cell wall-based components such as chitin synthase, Glucosamine-6-phosphate synthase, UDP-glucosyltransferase and Sterol 14α-Demethylase Cytochrome P450.

Methods

Collection of Plant Samples and Extraction of Essential Oil

Plant samples were collected from the Botanical Garden of the IGNTU Campus, Amarkantak, M.P., India. An amount of 250 g of fresh leaves was taken, cut into small pieces, and thoroughly washed with sterilized water. The volatile fraction was isolated by hydro-distillation through Clevenger's apparatus. The isolated fraction of the plant parts exhibited two distinct layers: an upper layer and a lower aqueous layer. Both layers were separated, and the essential oil was stored in clean glass tubes.

In-Vitro Antifungal Activity of Lemon Grass Essential Oil

Antifungal activities of essential oil were tested against the four phytopathogenic fungi, viz., *Fusarium oxysporum, Alternaria alternata, Aspergillus flavus,* and *Aspergillus niger*, following the poisoned food technique [15]. Throughout the whole experiment, potato dextrose agar medium (39 g of Hi-PDA medium dissolved in 1000 ml of distilled water) was used. While preparing the medium, 30 mg/L of streptomycin was added to prevent bacterial contamination. Different amounts of essential oil were dissolved separately in 0.5 ml of a 0.01% tween-20 aqueous solution. To evaluate the true effect of essential oils on the pathogenic fungi, tween 20 solvent care was taken. An amount of 9.5 mL of molten PDA was taken in each petri plate and mixed with the essential oil, which was earlier mixed with the 0.01% tween-20 solution, so as to obtain the requisite concentrations, viz., 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm, and 6000 ppm, respectively. Sterilized water instead of oil served as a control. With the help of a sterilized cork borer, a 5 mm-diameter disc of fungal mycelium was cut from the periphery of a 7--day-old culture and placed aseptically at the center of each petri plate of the control and treated sets. All the petri plates were incubated at 25 ± 2°C for seven days in a BOD incubator. After seven days, colony diameter was measured, and the percentage inhibition of mycelial growth was calculated using the following formula:

Percent mycelial inhibition = dc-dt/dc X 100

Where dt = mean colony diameter of treatment sets

dc = mean colony diameter of control sets

Minimum Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentration at which the oil showed absolute fungitoxicity (complete inhibition of the growth of test fungi), the usual poisoned food technique was implemented. Different concentrations of the essential oils were prepared by dissolving separately their requisite amount in 0.5 ml of 0.01% aqueous solution of tween-20 and then mixing with 9.5 ml of molten potato dextrose agar medium. The control sets were maintained with the requisite amount of sterilized water dissolved in 0.5 ml of tween-20 in place of oil. As usual, the prepared plates were inoculated upside down aseptically with the assay disc of the test fungi on the center of the petri plates of the treatment and control sets. The petri plates were incubated at 25 ± 2°C for seven days in a BOD incubator. Diameters of the fungal colonies of the treatment and control sets were measured in mutually perpendicular directions on the seventh day, and percentage inhibition was calculated.

Chemical Composition Analysis of Essential Oil

The chemical composition of the essential oil was analyzed using a Nexis GC-2030 GAS chromatograph system (Shimadzu, Kyoto, Japan) coupled with a flame ionization detector (FID) [16]. Initially, the oven temperature was set at 60°C, which was slowly raised to 250°C. Essential oil of 10 µL was added with 1 mL of petroleum ether, and 1 µL was injected into the column (SH-Rxi-5Sil MS) through split mode (1/10 ratio). Helium gas was used as a carrier gas with a flow rate of 1.2 ml/min. A unique

peak was detected by the MS detector (50 to 550 m/z). The compounds were identified on the basis of their retention time. The concentration of the compounds was determined by the peak area obtained. Finally, the spectral data of the peaks were analyzed by comparing them with the library data containing mass spectra (WILEY8 and NIST14). The relative percentage of each individual compound was compared through peak area normalization.

Molecular Docking

Ligand and Protein Preparation

GC-MS identified ligands were obtained in Structured Data File (SDF) format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and converted to PDBQT format with Open Babel software for molecular docking analysis. Chitin synthase (PDB ID: 4GF8), UDP-glucosyltransferase (PDB ID: 5U6M), Glucosamine 6-phosphate synthase (PDB ID: IJXA), and Sterol 14-Demethylase Cytochrome P450 (CYP51) (PDB ID: 5TZ1) three-dimensional structures were obtained in PDB format from Protein Databank (PDB). (https://www.rcsb.org/). Then, using BIOVIA Discovery Studio, heteroatoms such as water and cocrystalized ligands were eliminated. Swiss PDB viewer 4.10 was used to repair missing atoms in the amino acid chain. The active sites of each protein were identified using the Computed Atlas of Protein Surface Topography (CASTp) web server.

Docking Analysis

Autodock 4.2.6 was utilized for analysis, along with Python 3.9.6 and MGLTools 1.5.6. Protein-ligand docking was performed by binding one ligand at a time to the protein receptor. The processed protein was then imported into an Autodock workspace, where polar hydrogen atoms and Kollman charges were introduced [17]. Similarly, ligands were added to the same workplace for Gasteiger charge addition and to define its torsion tree by selecting a root and a rotatable number of bonds. The protein and ligands were both saved in PDBQT format for further use.

Preparation of Grid Parameter and Autodocking

Grid parameters were specified for all considered proteins to investigate the active site and ensure perfect ligand interaction. The center grid box values and grid points for both the receptor proteins in the x, y, and z dimensions are mentioned in Table 1. After successfully executing Autogrid, the genetic algorithm was set to the following parameters: (a) 50 GA runs; (b) 300 population size; (c) 2.5 million energy evaluations; and (d) 27,000 generations. Subsequently, the Lamarckian genetic process was employed, and the output file was saved in a docking parameter file (DPF). Finally, the docking program was initiated, and the final result was saved as a docking log file (DLG). The inhibition constant and binding energy were calculated and visualized in the discovery studio for 2D and 3D evaluation.

Table 1: Target protein grid parameter based on amino acid residue

Proteins	PDB ID	Center grid box	Grid points
Chitin synthase	4GF8	x=58, y=64, z=66	x=-2.845, y=-29.597, z=11.306
UDP-glucosyltransferase	5U6M	x=64, y=62, z=64	x=68.015, y=53.938, z=17.199
Glucosamine 6-phosphate synthase	IJXA	x=58, y=54, z=56	x=-14.234, y=0.745, z=5.117 x=58, y=64, z=66
Sterol 14α-Demethylase Cytochrome P450 (CYP51)	5TZ1	x=88, y=64, z=80	x=68.015, y=53.938, z=17.199

Results

In-Vitro Antifungal Activity of Lemongrass Essential Oil

The antifungal activity of Lemongrass essential oil was examined by calculating the percentage inhibition of radial mycelial growth of the tested fungi, viz. *Fusarium oxysporum, Alternaria alternata, Aspergillus flavus*, and *Aspergillus niger*, as shown in Figure 1. Figure 2 shows the percentage inhibition of mycelial growth of tested fungi at different concentrations (500 ppm, 1000 ppm, 2000 ppm, 4000 ppm, and 6000 ppm) of essential oil. At a concentration range of 1000–6000 ppm, 100± 00% of mycelial inhibition was recorded against *Alternaria alternata* and *Aspergillus niger*, whereas 100± 00% inhibition of the fungi *Fusarium oxysporum* and *Aspergillus flavus* resulted in a range of 2000–6000 ppm, respectively. At the lowest concentration (500 ppm) of essential oil, the maximum percentage of inhibition was found against *Alternaria alternata*, followed by *Fusarium oxysporum, Aspergillus flavus,* and *Aspergillus niger fungi*, respectively. The essential oil significantly inhibited the mycelial growth of all the tested fungi, as depicted in figure 2. The fungi-toxic nature of essential oil was found to be dose-dependent.

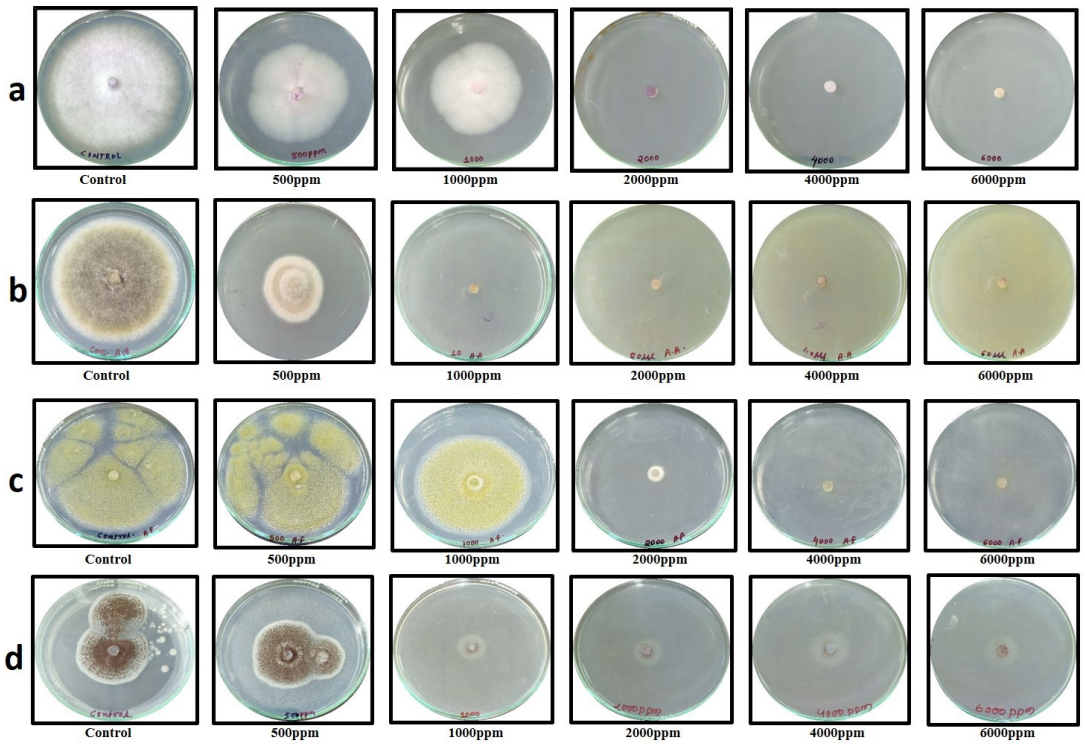

Figure 1: Effect of different concentration of essential oil against phytopathogenic fungi viz, (a) *Fusarium oxysporum*, (b) *Alternaria alternata*, (c) *Aspergillus flavus* and (d) *Aspergillus niger,* respectively

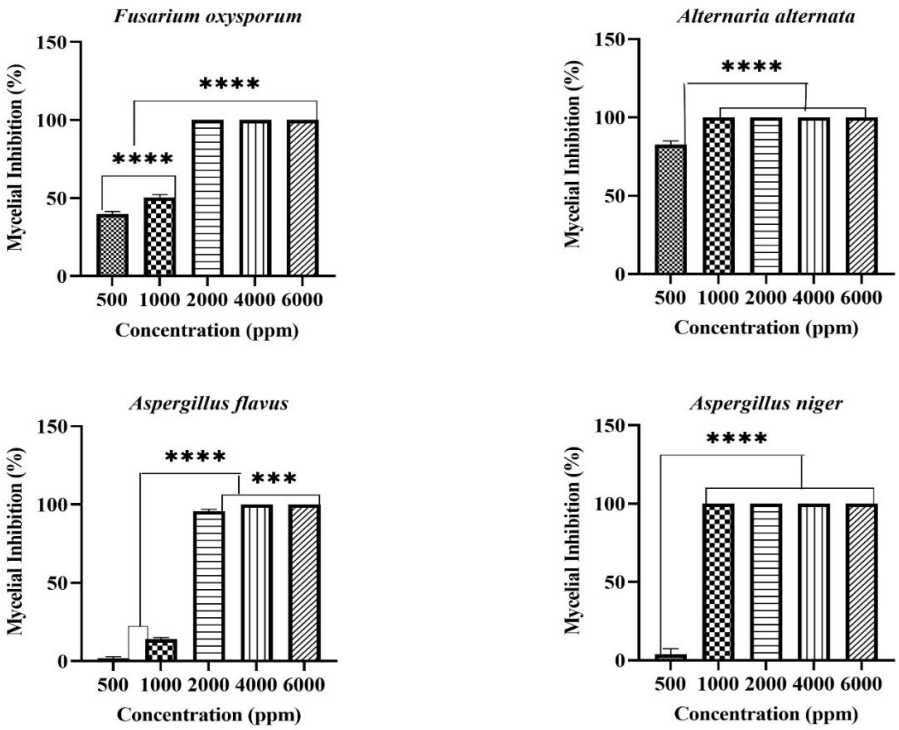

Figure 2: Percentage inhibition of mycelial growth of phytopathogenic fungi at different concentrations of Lemongrass essential oil. Asterisk *, **, *** & **** representing the significance difference between samples at p< 0.05, 0.01, 0.001 & 0.0001, respectively

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of essential oil against tested fungal pathogens showed effective results. At a concentration of 2000 ppm of essential oil, a fungitoxicity effect was found against *Fusarium oxysporum* and *Aspergillus flavus*, whereas at a lower concentration (1000 ppm), a fungitoxic effect resulted against *Alternaria alternata* and *Aspergillus niger* fungi, respectively, as depicted in Table 2.

Table 2: Minimum inhibitory concentration of Lemongrass essential oil against phytopathogenic fungi

Sl. No	Pathogenic fungi	MIC of oil against fungi
1	*Fusarium oxysporum*	2000ppm
2	*Alternaria alternata*	1000ppm
3	*Aspergillus flavus*	2000ppm
4	*Aspergillus niger*	1000ppm

Chemical Composition Analysis of Essential Oil

The chemical composition of the essential oil extracted from the *Cymbopogon citratus* plant is depicted in **Table 3**. The peaks were examined, and their respective retention times were also displayed (Figure 3). The GC-MS analysis of Lemongrass essential oil revealed 40 compounds. The GC-MS chromatogram contained three major peaks along with many small peaks, indicating the presence of major compounds. The major constituents of the oil were 2,6-Octadienal, 3,7-dimethyl- (E)- (Citral), Neral (Beta Citral), 2-Pentanone, 4-hydroxy-4-methyl- (Diacetone alcohol), Geraniol (Lemanol), and Geranyl acetate respectively.

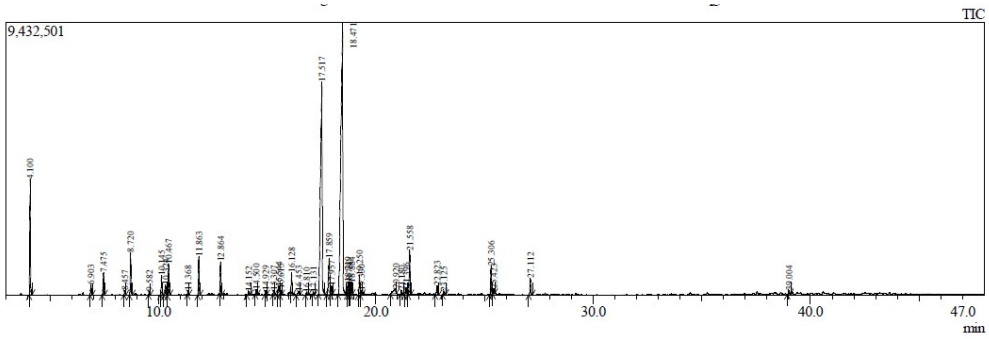

Figure 3: GC-MS chromatogram of Lemongrass essential oil based on their retention time

Table 3: Base mass of compounds in *C. citratus* oil

Peak#	R.T me	F.Time	Area	Area%	Height	Mark	Similarity	Base m/z	Name
1	4.1	4.195	9563952	6.18	3985703		0	43.05	2-Pentanone, 4-hydroxy-4-methyl-
2	6.903	6.965	924034	0.6	342031		0	93.15	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
3	7.475	7.54	2055907	1.33	766667		0	93.15	Camphene
4	8.457	8.51	349632	0.23	138629		0	93.15	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methyl
5	8.72	8.805	3802370	2.46	1471261		0	43.05	5-Hepten-2-one, 6-methyl-
6	9.582	9.635	302219	0.2	122368		0	93.15	3-Carene
7	10.145	10.205	1724333	1.11	673560		0	119.15	o-Cymene
8	10.324	10.38	957098	0.62	364881		0	68.1	D-Limonene
9	10.467	10.545	2842117	1.84	1070585		0	43.05	Eucalyptol
10	11.368	11.415	295413	0.19	125133		0	93.15	.gamma.-Terpinene
11	11.863	11.93	3479894	2.25	1322694		0	71.1	4-Nonanone
12	12.864	12.93	2866584	1.85	1130828		0	71.1	Linalool
13	14.152	14.25	275826	0.18	112663	MI	0	70.1	Cyclohexanone, 5-methyl-2-(1-methylethylide
14	14.5	14.56	490440	0.32	171903		0	123.2	cis-Chrysanthemyl alcohol
15	14.929	14.98	363397	0.23	149673		0	67.1	Isoneral
16	15.307	15.36	345135	0.22	134547		0	95.15	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1
17	15.544	15.58	976119	0.63	269105		0	67.1	3,6-Octadienal, 3,7-dimethyl-
18	15.615	15.675	578278	0.37	203481	V	0	84.1	Carveol
19	16.128	16.19	1733941	1.12	690164		0	148.15	Estragole
20	16.453	16.52	519449	0.34	129162	MI	0	84.1	p-Mentha-1(7),8-dien-2-ol
21	16.81	16.91	219091	0.14	76457	MI	0	81.1	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethen
22	17.131	17.2	181457	0.12	72292	MI	0	95.15	6-Octen-1-ol, 3,7-dimethyl-, (R)-
23	17.517	17.66	37666776	24.33	7342395		0	41.1	Neral
24	17.859	17.92	5458543	3.53	1250875		0	69.1	Geraniol

25	17.957	18.02	783836	0.51	298152	V	0	82.1	2-Cyclohexen-1-one, 3-methyl-6-(1-methyleth
26	18.471	18.66	57888169	37.38	9191700		0	69.15	2,6-Octadienal, 3,7-dimethyl-, (E)-
27	18.719	18.75	1106481	0.71	435178		0	59.1	Epoxy-linalooloxide
28	18.78	18.83	814326	0.53	320922	V	0	43.05	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-m
29	18.884	18.94	1010558	0.65	384346	V	0	59.1	Epoxy-linalooloxide
30	19.25	19.29	1667595	1.08	645980		0	69.1	2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (Z)
31	19.33	19.39	422681	0.27	156057	V	0	126.15	Isophytol, acetate
32	20.92	20.955	1413574	0.91	166756	MI	0	69.1	2,6-Octadienoic acid, 3,7-dimethyl-, (E)-
33	21.18	21.27	392118	0.25	131682		0	110.15	2-Hexenoic acid, 3,4,4-trimethyl-5-oxo-, (Z)-
34	21.396	21.47	812131	0.52	232659		0	105.15	1,2,4-Metheno-1H-indene, octahydro-1,7a-dim
35	21.558	21.63	4067694	2.63	1523664		0	69.1	Geranyl acetate
36	22.823	22.91	956332	0.62	290980		0	93.15	Caryophyllene
37	23.125	23.225	225310	0.15	84998	MI	0	83.1	Globulol
38	25.306	25.37	2710480	1.75	939917		0	161.2	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-m
39	25.423	25.47	466288	0.3	173872	V	0	161.2	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dime
40	27.112	27.21	1742297	1.13	572710		0	93.1	Caryophyllene oxide
41	39.004	39.09	391892	0.25	118472		0	69.1	2-Buten-1-one, 1-(2,2,5a-trimethylperhydro-1
			154843767	100	37785102				

In Silico Investigation of Antifungal Activity of GC-MS Identified Metabolites

To examine binding affinity, molecular docking was performed for four compounds against Chitin synthase, Glucosamine-6-phosphate synthase, UDP-glucosyltransferase and Sterol 14α-Demethylase Cytochrome P450. Virtual screening technologies are often and widely used as a strategy for discovering novel ligands for protein structures. Binding energy and inhibition constants were calculated for each compound with target proteins (Figure 4). The highest binding affinity (−7.11 Kcal/mol) was exhibited by geranyl acetate against chitin synthase, with a lower inhibition constant (6.19 μM). Next, it showed good binding with Sterol 14α-Demethylase Cytochrome P450 (-6.96) and UDP-glucosyltransferase (−6.37 Kcal/mol) with 7.97 μM and 21.55 μM inhibition constants (Ki). Although it is observed that geranyl acetate with each targeted protein has exhibited the highest binding affinity compared with the other three compounds, in case of geraniol, good binding affinity was observed with chitin synthase (-6.23) and Sterol 14α-Demethylase Cytochrome P450 (-6.53 kcal/mol). Among all tested compounds, 2-Pentanone, 4-hydroxy-4-methyl, has shown less binding affinity with each protein, such as chitin synthase (-5.36), Glucosamine-6-phosphate synthase (-4.95), UDP-glucosyltransferase (-5.15), and Sterol 14α-Demethylase Cytochrome P450 (-5.82 Kcal/mol). Further, the structural interaction studies for bond formation were visualized in Discovery Studio. Geranyl acetate was able to form a maximum of three hydrogen bonds with Asn29 (two bonds) and the Gln462 residue of chitin synthase (Figure 5). In the case of geraniol, it shared two hydrogen bonds with chitin synthase (Ser128 and Tyr132) and UDP-glucosyltransferase (Phe271 and Gly272). Likewise, citral with glucosamine-6-phosphate synthase made three hydrogen bonds (Cys300, Thr355, and Thr352). 2-Pentanone, 4-hydroxy-4-methyl, shared the maximum four hydrogen bonds with glucosamine-6-phos-

phate synthase.

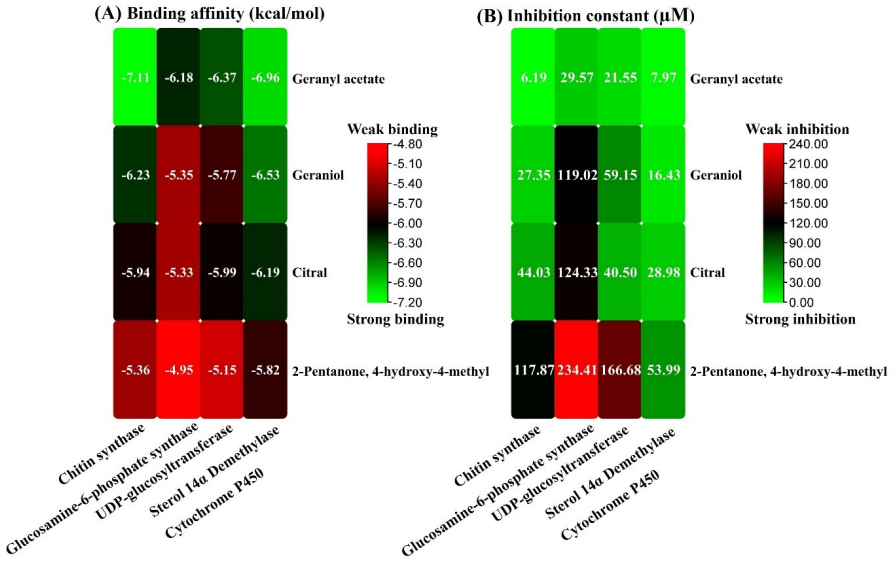

Figure 4: Binding energy and inhibition constant of Lemongrass identified metabolites against different target proteins

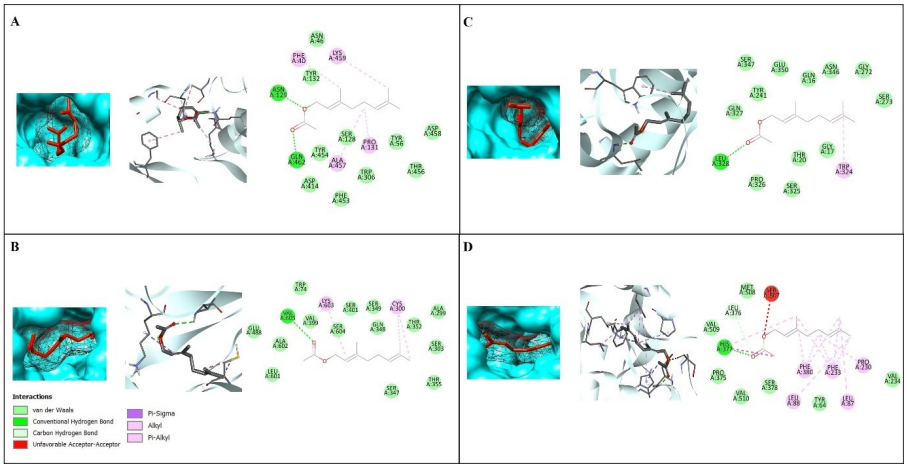

Figure 5: Molecular docking interaction showing 2D, 3D and surface view of geranyl acetate with (A) Chitin synthase, (B) Glucosamine-6-phosphate synthase, (C) UDP-glucosyltransferase, and (D) Sterol 14α-Demethylase Cytochrome P450

Discussion

The widespread application of chemical pesticides in agro-ecosystems has significant drawbacks like handling hazards, increased cost, food contamination, and threats to human health and the environment [18]. To overcome such issues, natural plant-based protectants with pesticidal activity as well as their low mammalian toxicity and less environmental impact gained wide public acceptance. The essential oil of Lemongrass was found to inhibit the growth of all tested phytopathogenic fungi. The mycelial growth of fungi Alternaria alternata and *Aspergillus niger* was restricted 100% at 1000 ppm concentrations of essential oil, whereas fungi *Fusarium oxysporum* and *Aspergillus flavus* were restricted 100% at 2000 ppm. Results indicate that Lemongrass essential oil has a significant fungitoxicity effect against phytopathogenic fungi. Antifungal activity of the essential oils of *Artemisia nilagerica, Litsea cubeba*, and *Mikania cordata* has reported against the phytopathogenic fungi *Alternaria alternata, Botrytis cinera, Fusarium oxysporum*, and *Penicillium expansum* and observed a fungicidal effect at a concentration of 1000 ppm [8]. The 100% reduction of fungal growth at a 500ppm concentration of Lemongrass essential oil was reported against the phytopathogenic fungi *Colletotrichum coccodes, Botrytis cinera, Cladosporium herbarum, Rhizopus stolonifers,* and

Aspergillus niger [19]. In this study, dose-dependent mycelial growth inhibition was observed against all the tested fungal pathogens. Similarly, the antifungal activity of different concentrations of Lemongrass essential oil reported to tested against phytopathogenic fungi such as *Alternaria alternata, Botrytis cinera, Fusarium oxysporum, Pythium ultimum,* and *Rhizoctonia solani* resulted 100% reduction at a concentration of 1.0%, while decreasing the concentration to 0.5%, 0.25%, and 0.1% resulted in a percentage inhibition of 65%, 60%, and 0% against *Alternaria alternata*, 70%, 0%, and 0% against *Botrytis cinera,* 100%, 62%, and 57% against Fusarium oxysporum, 50%, 0%, and 0% against *Pythium ultimum*. But at lower concentrations of essential oil, effect on the radial growth of *Rhizoctonia ultimum* was not observed [20].

The essential oil of Lemongrass and its associated compounds exhibited good antifungal potential. The IC_{50} and IC_{90} values of the essential oil were found at 0.229 µl/mL and 0.358 µl/mL against the fungus *Fusarium avenaceum*, whereas the individual compounds such as citral, β-citronellol, geraniol, geranly, acetate, (±)-citronellal, and limonene exerted stronger antifungal effects, with IC_{50} values of 0.087 µl/mL, 0.141 µl/mL, 0.326 µl/mL, 0.575 µl/mL, 0.705 µl/mL, and 2.797 µl/mL, respectively [21]. The essential oil of Lemongrass has been reported to be effective against phytopathogenic fungi as well as human pathogenic fungi such as, *Colletotrichum acutatum* and *C. gloeosporioides*[22], *Candida albicans*, *Candida auris*, *Candida famata*, *Candida krusei*, and *C. tropicalis* [14].

The chemical composition analysis of Lemongrass essential oil resulted five major constituents of monoterpenes, such as 2,6-Octadienal, 3,7-dimethyl-, (E)- (Citrol), Neral (Beta Citrol), 2-Pentanone, 4-hydroxy-4-methyl- (Diacetone alcohol), Geraniol (Lemanol), and Geranyl acetate. Essential oils associated with different plant species mainly contain alcohols, terpenes, phenols, aldehydes, and terpenoids, which have been reported to have good fungicidal, antimicrobial, nematocidal, and insecticidal activities [23]. In this study, Lemongrass essential oil included 2,6- Octadienal, 3,7-dimethyl-, (E) (citrol), Neral (Beta citrol), 2- Pentanone, 4-hydroxy-4-methyl (Diacetone alcohol), Geraniol and Geranyl acetate, and the content of citrol was higher than that of the other compounds. Myrcene and methyl iso-eugenol were the major compounds of the essential oil in leaf Jor Lab L-11 of Lemongrass (*Cymbopogon flexuosus*); however, citral-A, citral-B, geraniol, geranyl acetate, β-citronellol, citronellal, and limonene, respectively, were the minor compounds in the essential oils [24]. Among the many commercially produced essential oils from numerous plants, Lemongrass essential oil extracted from the *Cymbopogon* species is gaining wide acceptance.

In order to predict the binding affinity of essential oil metabolites, molecular docking was performed against fungal proteins. Numerous studies indicate that molecular docking analysis has predicted several natural inhibitors against pathogen-essential proteins to screen the effective ligands. However, our study suggested the geranyl acetate as an excellent inhibitor against each target protein especially with chitin synthase. A previous study revealed that geranyl acetate has been found to be an effective inhibitor against the cell wall synthesis protein of Methicillin-Resistant *Staphylococcus aureus* and Multi-Drug Resistant *Pseudomonas aeruginosa* [25]. Likewise, citral is well known antimicrobial agent from *Cymbopogon* spp. It is present in leaves and fruits of various plant species, such as myrtle trees, basil, lemon, lime, lemongrass, orange, and bergamot, and frequently found in the form of stereoisomers of geranial and neral [26, 27]. Previously, reported that *C. citratus* essential oil showed potent antifungal activity against *A. fumigatum* and possesses significant binding affinity of citral with chitin synthase, UDP-glycosyltransferase and Glucosamine-6-phosphate synthase [28]. Furthermore, the inhibitory activity of geraniol has been reported against *A. flavus* and *A. ochraceus* concerning their cell membrane permeability, reactive oxygen species (ROS) generation, and growth-related gene expression [29]. The proteins targeted in this study are the essential protein of fungus and their inhibition through metabolites identified in the essential oil of *C. citratus* could be a possible reason of their antifungal activity against all tested fungi under *in vitro* study.

As modern agricultural system has been coping with the use of hazardous agrochemicals, raising health risk as well as degrading natural ecosystem. There is an urgent need to overcome such scenario that may possibly be manoeuvre by use of botanical fungicides. Through our experimental evidence it concluded that lemon grass essential oil has a potential fungicidal effect

against tested phytopathogens, hence it could be used as a botanical fungicide. The major challenges associated with essential oil is environmental conditions as well as farmers response and acceptability. This finding could help to develop botanical fungicides over chemical-based fungicides, can add an alternative strategy for fungal disease management in agricultural field.

Conclusion

Phytopathogenic fungi are the major causal agent responsible for about 70–80% of the plant's disease, adversely affecting crop growth and yield. This study reports the chemical composition analysis of *C. citratus* essential oil and their *in vitro* as well as *in silico* antifungal activity against the major phytopathogenic fungi, viz., *F. oxysporum, A. alternata, A. flavus,* and *A. niger*. The GC-MS analysis revealed the presence of 40 compounds; among them, the major constituents of the oil were 2,6-Octadienal, 3,7-dimethyl- (E)- (Citral), Neral (Beta Citral), 2-Pentanone, 4-hydroxy-4-methyl- (Diacetone alcohol), Geraniol (Lemanol), and Geranyl acetate. *In vitro* antifungal activity of essential oil showed a significant percentage of mycelial inhibition against all the tested phytopathogenic fungi. The MIC for fungi *A. alternata* and *A. niger* resulted at 1000 ppm, whereas fungi *F. oxysporum* and *A. flavus* resulted at 2000 ppm. Furthermore, using molecular docking analysis, metabolites predicted in essential oil were investigated for their binding affinity and inhibition constant, showing geranyl acetate to be an effective inhibitor against various fungal proteins. The results showed that geranyl acetate was a potent inhibitor of several fungal proteins. This work was a preliminary step towards identifying the constituents of *C. citratus* essential oil and their *in vitro* and in silico studies to prevent phytopathogenic fungi, which may then be applied to sustainable agriculture practices.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Purusottam Majhi, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

Authors do not have any conflict of interest. The authors do not have affiliations with or involvement in any organization or entity with any financial interest or in the subject matter or materials discussed in this manuscript

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