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INSILICO SCREENING OF BIOCOMPONENTS IN *CYMPOBOGAN CITRATUS* AND *ALLIUM SATIVUM* AGAINST *MICROSPORUM SP*

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ABSTRACT

Effective and nontoxic antifungal agents are required for the treatment of dermatophytosis. Plants and their extracts have been used as medicines against superficial infections. In our present study, from the Leaves of *Cymbopogon citratus* (lemon grass) and pulp of *Alllium sativum* (garlic) extracts were prepared by using different solvent i.e., Ethanol, Ethyl acetate, Chloroform and Aqueous extract using soxhlet apparatus and to investigate the antifungal activity. Water Samples were collected from sewage, swimming pools and lakes in Chennai, Tamilnadu. *Microsporum gypseum* and *Microsporum canis* were isolated and identified from the samples. Phytochemicals analysis with the Leaves of *Cymbopogon citratus* (lemon grass) and pulp of *Alllium sativum* (garlic) extracts were studied using different solvent i.e., Ethanol, Ethyl acetate, Chloroform and Aqueous extract MIC and MFC were carried out with the isolates, *Cymbopogon citratus* extract with Ethyl acetate showed higher antidermatophytic activity, by inhibiting the *Microsporum canis* at the concentration of 62.5(µg/ml), and Ethanolic extracts the concentration of 125(µg/ml) and aqueous extract in the concentration of 500(µg/ml). *Alllium sativum* extract *with* Ethyl acetate showed higher antidermatophytic activity,by inhibiting the *Microsporum gypseum* at the concentration of 62.5(µg/ml), and chloroform extract in the concentration of 250(ug/ml) and aqueous extract in the concentration of 1000(µg/ml). Gas Chromatography Mass Spectroscopy was done to identify the biochemical peak components and the activities present in the *Cymbopogon citratus* and *Alllium sativum* extracts. Seven compounds were identified with their following retention time. Citral (18.83), dimethyl sulfide (19.58) followed by nonadeconic acid (20.87), Brassidic acid (21.87), Behenic acid (22.82), hexodeconic acid (17.17) and Flavone (14.07). of which Flavone, citral and dimethyl sulphide acts as the ligands to bind with the specific proteins present in the organism and to have inhibitory activity. Molecular docking was done for the LAP1 protein and MEP1 proteins from *Microsporum sp*. Citral compound found to have greater inhibitory property against the pathogenic dermatophytes. Ethyl acetate extracts of *Cymbopogon citratus* and *Alllium sativum* have medicinal property to treat infectious pathogenic fungi.

KEY WORDS: ***Dermatophytes, Medicinal plants, phytochemical analysis, Microsporum gypseum, Microsporum canis, GC-MS, Molecular docking.***

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INTRODUCTION

Soil is considered as one of the most complex microbial habitats in which many fungi complete their life. Soil serves as a natural reservoir for both pathogenic and saprophytic fungi. Factors influencing the distribution of keratinophilic fungi have been relatively well recognized in the soil environment.[1] The occurrence of fungi in the soil is influenced by biological and non-biological factors, such as soil, pH, temperature, humidity, environmental light, climate, chemical composition and amount of organic material in the soil.[2] Dermatophytosis is one of the most infectious diseases of humans, caused by invasion of stratum corneum by dermatophytic fungi viz. *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum* spp, *Epidermophyton* spp.[3] and remains a common public health problem, especially in tropical countries such as India.[4] *Trichophyton mentagrophytes* is considered as a zoophilic and anthrophilic dermatophyte [5,] which causes hair, skin and nail infections in humans. The most frequent species, such as: *Trychophyton spp., Microsporum spp., Epidermophiton spp.* and *Candida spp*., has been observed. There are about 16 valid species belonging to the genus *Microsporum* which are associated with the skin and hair infections . However, they are not associated with nail infections. *Microsporum audouinii* is the prototype of this genus.[6] The shape of macroconia varies from spindle or fusiform to obovate (egg shaped) in *M. nanum* and cylindrofusiform in *M. vanbruseghemii.*. Macroconida may be septate having 1-15 septa, the size of which may vary from 6µm-160µm by 6µm-25µm. Some of the commonly observed species of *Microsporum* are; *M. audouinii M. canis, M. gypseum, M. nanum, M. ferrugineum, M. cookie, M. vanbreuseghemii, M. persicolor.*The work of David Gruby laid down the foundation of dermatomycology. He first described the clinical entity caused by dermatophytes and also demonstrated their contagious nature. He also recognized ectothrix and endothrix hair invasion of a dermatophyte species and named it as *Microsporum audouinii* . Raymond Sabouraud, a renowned mycologists initiated work on dermatophytosis According to the host and the fungal species involved, the typical aspect of dermatophytic lesions may be modified. A few antifungal agents are available and licensed for use in veterinary practice or human being treatment. The use of systemic drugs is limited to treat man or animal due to their high toxicity and problems of residues in products intended for human consumption.[7] Different treatments have been recommended to control dermatophtes. In general, pharmacological treatment option includes antifungal agents[8], but recently the use of some natural plant products has been emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented since the late 19th century.[9] These natural plants involve garlic, lemon grass, datura, acacia, a triplex, garlic, black seed, neem, basil, eucalyptus, alfalfa and basil . They are safe to human and the ecosystem than the chemical antifungal compounds, and can easily be used by the public who used them for thousands of years to enhance flavor and aroma of foods as well as its economic value. [10] Lemon grass *(Cymbopogon citratus*) belongs to the family *Poaceae*. It is a tall perennial grass with slender sharp edge green leaves that have a pointed apex. It is a native to warm temperate and tropical regions. It is used as perfumes in soap, creams, candles and detergents. Lemon grass tea can be used to treat fever, cold, cough and stomach upset. The tea has diuretic properties and water retention, making it helpful in individual with high blood pressure. It can also help to prevent typhoid fever, cancer and blurring of vision. It can help to relieve menstrual problems and nausea. The lemon grass can help to lower cholesterol levels. Lemon grass can be used in herbal medicine to treat nervous condition and inflammation. It can also be used to treat chest infections, sores, muscle cramps and headache.[11] Garlic *(A. sativum)* is an important food ingredient for many countries, especially in Asia. It is one of the herbs that have a lot of scientific report about its antibacterial and antifungal properties which comes from the substance called *allicin* (allyl 2-propene thiosulphinate). *Alicin* inhibits various thiol-dependent enzymatic systems of bacteria. It is one of the active ingredients found during crushing garlic. The use of higher plants and their extracts to treat infections is an ancient practice in traditional medicine. Human are using natural products of animals, plants and microbial sources for thousands of years either in the pure forms or crude extracts. About 80% of individuals from developed countries use traditional medicine, with origin from plants. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be investigated to better understand their properties, safety and efficacy .[12] Traditional medical practice has been continued for centuries in almost in every part of the world .[13] Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated .Gas chromatography-mass spectrometry (GC-MS) has been the most applied analytical techniques for essential oil analysis followed by the supercritical fluid extraction-gas chromatography,[14] Due to the complexity of essential oil compositions, sophisticated instruments such as high performance liquid chromatography in combination with gas chromatography (HPLC-GC),[15] are the preferred analysis. Auto Dock is a suite of automated docking tools. The software is used for modelling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structures. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools are used to prepare, run and analyze the docking simulations, in addition to modeling studies. Auto Dock is the most cited docking software because it is very fast, it provides high quality predictions of ligand conformations and good correlations between inhibition constants and experimental ones.

MATERIALS AND METHODS

Sample collection
About 60 Water Samples were collected from sewage, swimming pools and lakes in Chennai.

Isolation and identification
The samples were serially diluted and were plated onto Sabouraud dextrose agar plate with chloromphenicol to minimize bacterial contamination and cyclohexamide to reduce the contamination of saprophytic fungi. The inoculated sabouraud dextrose agar plates were incubated at room temperature for 21days.

Collection of plant materials
Cymbopogon citratus and *Allium sativum* are the two plant species selected to perform this experiment and were collected from Chennai. These two plants species were identified and authenticated from the institute of "Plant anatomy research centre" by the botanist Dr.P.Jayaraman.

Preparation of piant extract
The fresh leaves of *Cymbopogon citratus* and pulp of *Allium sativum* were washed to remove foreign materials like soil and dust. Leaves were dried under shade at room temperature without direct exposure to sunrays. It was then coarsely grounded by using mechanical device. Solvent extracts were prepared by soaking 10gm of powder successively in 100ml of ethanol, ethyl acetate, chloroform and distilled water. The extracts were stored at 4°C in closed container. The extract was prepared in soxhelet apparatus using three solvents such as ethanol, ethylacetate and chloroform and water.

Phytochemical analysis of cured extract
The extracts were subjected to different chemical tests for the detection of different phytoconstituents using standard procedures,[16]Extracts were tested for the presence of active compounds such as Tannins, Steroids, Flavonoids, Alkaloids, Steroids, Proteins, Phenol, Amino acids, and Carbohydrates.

Determination of Minimum Inhibitory Concentration (MIC)
Minimum Inhibitory Concentration (MIC) is lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism following overnight incubation, usually reported as mg/L. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the invitro activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints.
MIC was determined by adding 1ml of Sabouraud dextrose broth in 6 tubes. One ml of plant extract (*Cymbopogon citratus* and *Allium sativum)* was added in the first tube which gives the concentration of 1000µg/ml. It was further diluted until the concentration lowers to 31.25µg/ml respectively. Then 20µl of fungal culture (*Microsporum gypseum* and *Microsporum nanum*) was added to each tube and inoculated at room temperature for 21 days. Controls were also maintained.

Determination of Minimum Fungicidal Concentration (MFC)
The minimal fungicidal concentration (MFC) was defined as the concentration of antifungal agent at which the number of colony forming units was zero. (Approximately 99 to 99.5% killing activity) Minimum fungicidal concentrations were determined for each isolate medium drug combination.

Gcms
To identify the bioactive compounds in the extracts, it was further analysed by GC/MS machine after insertion into the GC-MS inlet port in a GC-MS vial. The results were printed out from the computer system connected to the GC/MS machine 14. The GC-MS is composed of two major building blocks; The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute from the column at different times called the retention time, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. MS identifies substances by electrically charging the specimen molecules, and accelerated them through a magnetic field, the molecules breaked into charged fragments and different charges are detected. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

Molecular docking
With the rapidly increasing amount of molecular biological data available, the computer-based analysis of molecular interactions becomes more and more feasible. Methods for computer-aided molecular docking have to include a reasonably accurate model of energy and must be able to deal with the combinatorial complexity incurred by the molecular flexibility of the docking partners. In both respects, recent years have seen substantial progress.[17].

RESULT

Sixty different water samples were collected and examined for dermatophytes from different places in chennai which included sewage water, swimming pool, pond water and river water. Out of the 60 samples 19 were isolated as *Microsporum canis*, 18 as *Microsporum gypseum,* 9 as *Microsporum nanum*, 6 as *Microsporum persicolor* and 4 were isolated on SDA medium as *Microsporum audouinii* Table1. Table 2 shows the result of phytochemical screening on *Cymbopogon Citratus* and *Allium sativum* showed that seven active ingredients were present in leaf and pulp. These include Tannins, Flavonoids, and Phenols, alkanoids, saponins, tannins proteins and free amino acid. Ethanolic extract of lemongrass leaves inhibited by *Microsporum* species in the concentration of 250 (µg/ml), whereas by ethyl

acetate extract in the concentration of 62.5 (µg/ml) and the aqueous extract in the concentration of 1000 (µg/ml) Table 3. *Microsporum* species was inhibited by chloroform extract of garlic bulb in the concentration of 250 (µg/ml), and ethyl acetate extract in the concentration of 125 (µg/ml), aqueous extract in the concentration of 500 (µg/ml) Table 4. Since the ethyl acetate extract of lemon grass and garlic showed least MIC and MFC against *Micropsorum* species, combined activity of plant by ethyl acetate extract is also tested and results are shown in Fig. 1. *Microsporum* species was inhibited by combined activity of ethyl acetate extracts of *Cymbopogon citratus* and *Allium sativum* in the concentration of 62.5 (µg/ml) Table 5 (Fig. 1). Graph 1 shows the analysis of sample (cymbopogan citratus and Allium sativum) was carried out and important constituents were identified with the help of GCMS. Seven compounds were identified with their retention time citral(18.83),dimethyl sulfide(19.58)followed by nonadeconic acid(20.87),Brassidic acid(21.87), Behenic acid(22.82), hexodeconic acid(17.17)and Flavone(14.07). Interaction between atoms of the ligands from *Microsporum gypseum* and the amino acid residues of LAP1 protein along with the hydrogen bond distance and docking score Table 6. Interaction between atoms of the ligands from *Microsporum canis* and the amino acid residues of MEP1 protein along with the hydrogen bond distance and docking score Table 7.

Table 1
Isolation of microsporum sp from water sample

S.NO	SPECIES	SEWAGE	SWIMMING POOL	POND	LAKE	TOTAL
1	*M.canis*	7	6	2	4	19
2	*M.gypseum*	8	6	3	1	18
3	*M.nanum*	4	3	1	1	9
4	*M. audounii*	3	2	1	-	6
5	*M. persicolor*	3	1	-	-	4

Table 2
Phytochemical studies on allium sativum and cymbopogon citrates

S.NO	Plant Constituents	Ethyl Acetate Extract
1	Alkaloids	-
2	Glycosides	+
3	Flavonoides	+
4	Saponins	+
5	Lignans	-
6	Tannins	+
7	Proteins	+
8	Free Aminoacid	+
9	Carbohydrates	-
10	Phenol	+

The above table shows the phytochemicals in combined activity of Allium sativum a nd Cymbopogon citrates with Ethyl acetate extract.

Table 3
Mic and mfc of allium sativum

S.No	Organism	Isolates	Ethyl acetate (µg/ml)		Chloroform (µg/ml)		Aqueous (µg/ml)	
			MIC	MFC	MIC	MFC	MIC	MFC
1.	*M.gypseum*	17	125	125	250	250	500	500
2.	*M.canis*	15	62.5	62.5	250	250	500	500

Table 4
Mic and mfc of cymbopogon citratus

S.No	Organism	Isolates	Ethyl acetate (µg/ml)		Ethanol (µg/ml)		Aqueous (µg/ml)	
			MIC	MFC	MIC	MFC	MIC	MFC
1.	*M.gypseum*	17	62.5	62.5	250	250	1000	1000
2.	*M.canis*	15	125	125	250	250	1000	1000

Table 5
MIC and MFC of Allium sativum and cympobogon citratus

S.No	Organism	Ethyl Acetate (µg/ml)	
		MIC	MFC
1.	*M.gypseum*	62.5	62.5
2.	*M.canis*	62.5	62.5

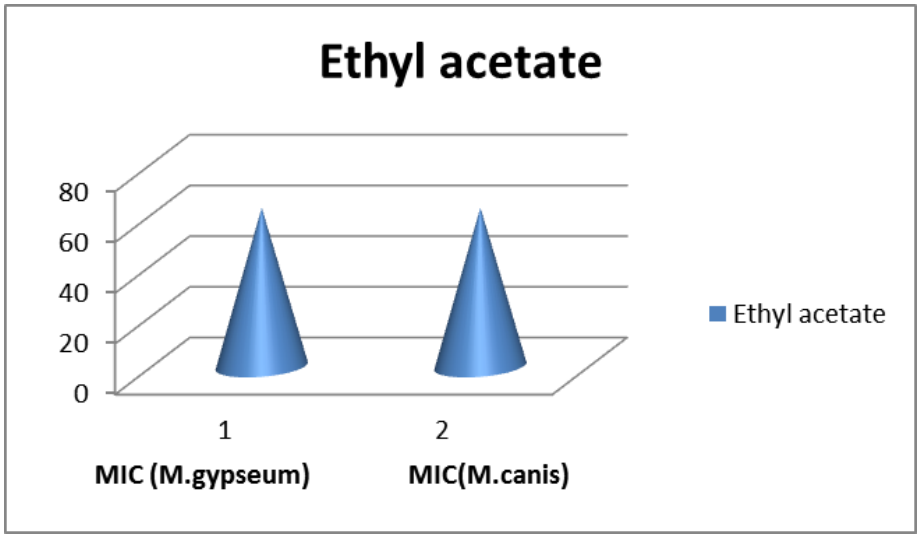
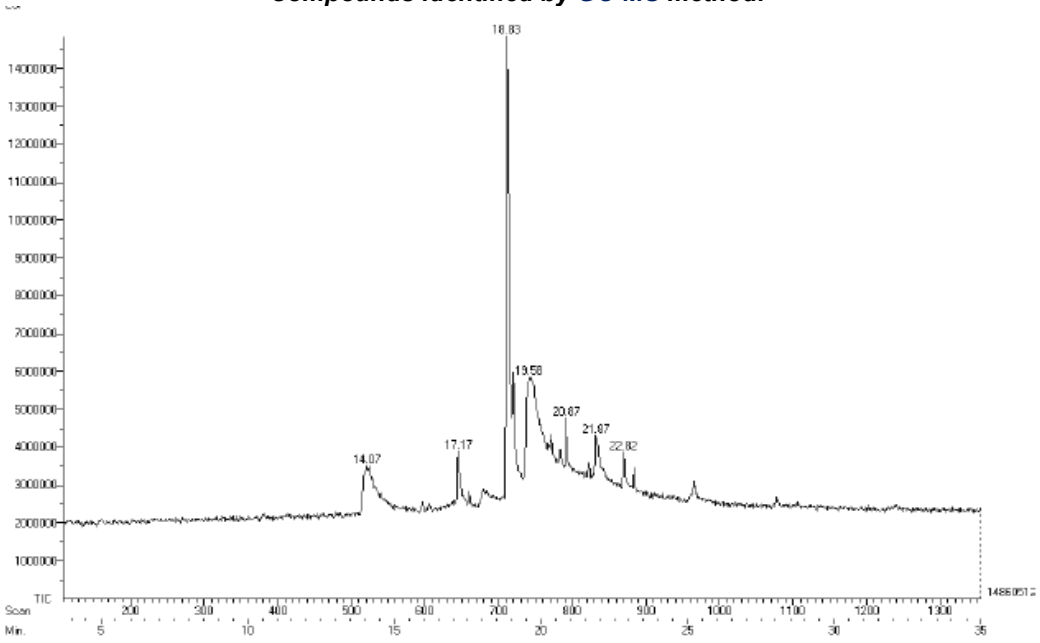


Figure 1
MIC of combined ethyl acetate extracts of Allium sativum and cymbopogon citratus

Graph 1
Compounds identified by GC-MS method.



Peak Report TIC

Peak No.	RT (Min.)	Compound Name	Peak Area	Peak Area (%)
1	14.07	Flavone	3507568	8.54
2	17.17	Hexadecanoic acid, methyl ester	3886048	9.46
3	18.83	Citral	14860512	36.18
4	19.58	Dimethyltrisulfide	5835552	14.21
5	20.87	Nonadecanoic acid, 18-oxo-, methyl ester	4785584	11.65
6	21.87	Brassidic acid	4315536	10.51
7	22.82	Behenic acid, methyl ester	3878656	9.44
		Total	**41069456**	**100.00**

Table 6
Leucine aminopeptidase 1(lap1)
***Microsporum gypseum* for 3d and 2d structure of ligands**

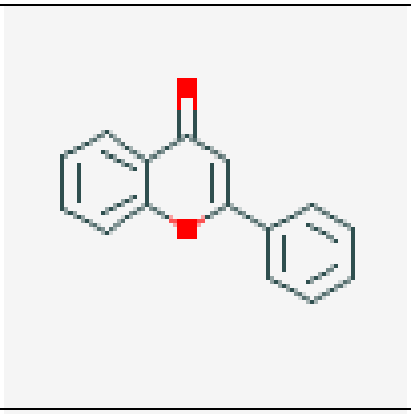
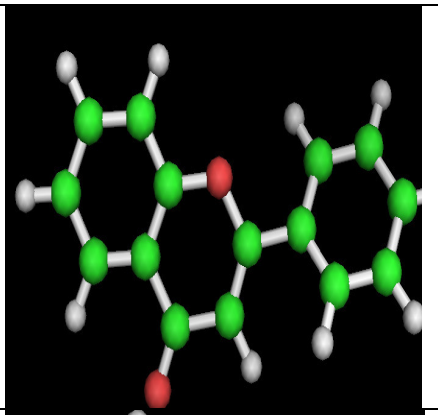
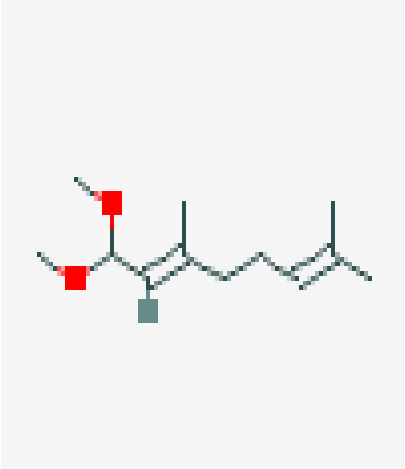
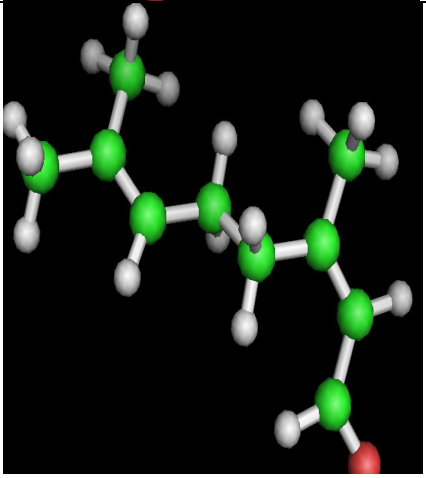
Compounds	2D structure	3D structure
2 phenychromone-4-ONE		
1,1-dimethoxy-3,7-dimethylocta-2,6-diene		

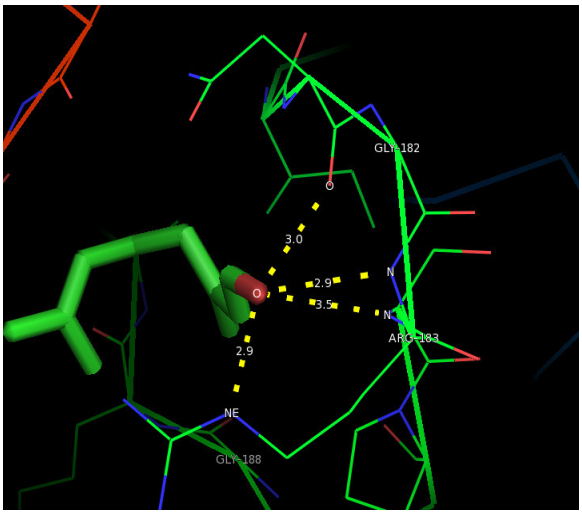
Table 6 (a)
Interaction between atoms of the ligands from Microsporum gypseum and the amino acid residues of LAP1 protein along with the hydrogen bond distance and docking score

Ligand	LAP1		Ligand Atom	Distance (Å)	Docking score (Kcal/mol)
	Residue	Atom			
2phenychromone-4-ONE	ALN 372	O	O	3.0	-7.51
1,1-dimethoxy-3,7-dimethylocta-2,6-diene	ASN181	O	O	3.0	
	GLY182	N	O	2.9	-4.57
	ARG183	N	O	3.5	
	SER 184	NE	O	2.9	

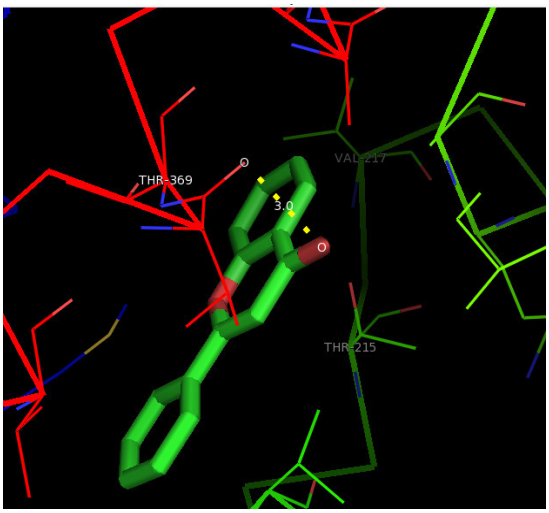
Table 6(b)
Shows key residues of LAP1, number of hydrogen bonds and docking score.

Compound	Key residues of LAP1	No. of hydrogen bond	Docking Score (Kcal/mol)
2phenychromone-4-ONE	ALN 372	1	-7.51
1,1-dimethoxy-3,7-dimethylocta-2,6-diene	ASN181 GLY182 ARG183 SER 184	4	-4.57

Figure 2
Docking of lap1



a) 2phenychromone-4-ONE



b) 1, 1-dimethoxy-3,7-dimethylocta-2,6-diene

Table 7
Extracellular metalloproteinase 1 (mep1)
***Microsporum canis* for 3d and 2d structure of ligands**

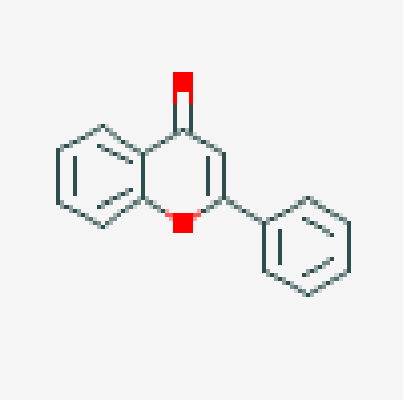
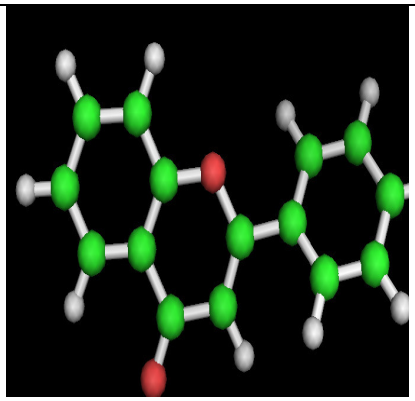
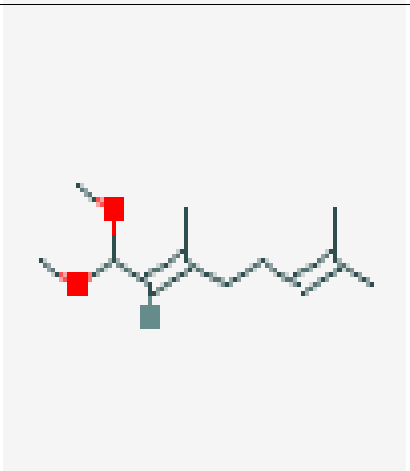
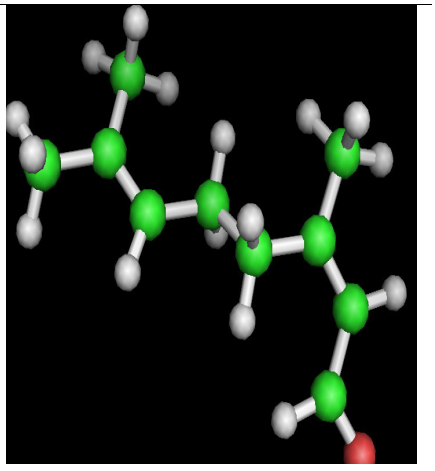
Compounds	2D structure	3D structure
2 phenychromone-4-ONE		
1,1-dimethoxy-3,7-dimethylocta-2,6-diene		

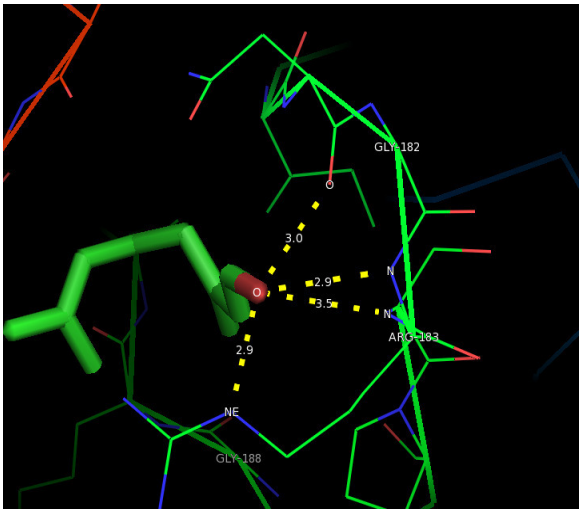
Table 7(a)
Interaction between atoms of the ligands from Microsporum canis and the amino acid residues of MEP1 protein along with the hydrogen bond distance and docking score

Ligand	MEP1		Ligand Atom	Distance (Å)	Docking score (Kcal/mol)
	Residue	Atom			
2phenychromone-4-ONE	ASN236	N	O	3.0	-7.04
	VAL237	ND2	O	3.2	
	VAL238	O	O	3.2	
	ASP239	N	O	3.1	
1,1-dimethoxy-3, 7-dimethylocta-2,6-diene	LEU40	OD1	O	3.3	-4.06
	GLY 41	N	O	3.1	

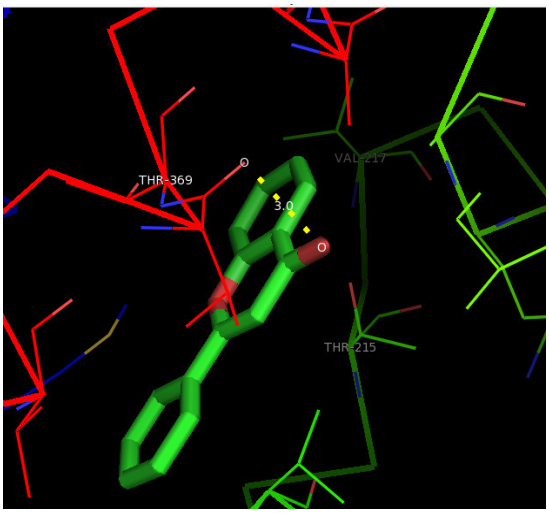
Table 7(b)
Shows key residues of MEP1, number of hydrogen bonds and docking score.

Compound	Key residues of MEP1	No. of hydrogen bond	Docking Score (Kcal/mol)
2phenychromone-4-ONE	ASN 236 VAL 237 VAL 238 ASP 239	4	-7.04
1,1-dimethoxy-3, 7-dimethylocta-2,6-diene	LEU 40 GLY 41	2	-4.06

Figure 3
Docking of lap1



A) 2phenychromone-4-one



B) 1,1-dimethoxy-3,7-dimethylocta-2,6-diene

DISCUSSION

This study is a stage in an investigation designed to evaluate the distribution of dermatophytes and other closely related keratinophilic fungi in the water samples. It was preceded by surveys of dermatophyte distributions in sewage water, pond water and river water. Out of 60 samples collected, 40 species of keratinophilic and non keratinophilic were isolated.Isolates like *Microsporum canis, Microsporum gypseum, Microsporum nanum*, *Microsporum persicolor* and *Microsporum audonii*. Table 1.The total of 19 isolates of *Microsporum canis*, 18 isolates of *Microsporum gypseum*, 9 isolates of *Microsporum nanum* and 6 of *Microsporum persicolor* was isolated from different water samples. [18]also isolated different species of microsporum from different water samples. The present study revealed that the crude extracts of the tested plants exhibited varied bioactivities against the examined dermatophytic and opportunistic fungal isolates Table 2. This Include Tannins, Flavonoids, and Phenols, alkanoids, saponins, tannins proteins and free amino acid. Extraction and Phytochemical screening of bioactive agents from medicinal plants permits the demonstration of their physiological activities. According to [19] tannins and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection. According to the report of [20], phytochemical component has antifungal properties which were confirmed in this study. The presence of tannins in the plant extract agrees with the report of ,[21] that tannins

are important in herbal medicine and they are applied in arresting bleeding and wound healing. Isolated *Microsporum canis, Microsporum gypseum, and Microsporum nanum*. *Microsporum gypseum* was inhibited by chloroform extract in the concentration of 250 (µg/ml), whereas by ethylacetate extract in the concentration of 125 (µg/ml) and the aqueous extract in the concentration of 500 (µg/ml). Ethyl acetate extract was found to have higher activity than the chloroform and aqueous extract against both the organism *Microsporum canis* and *Microsporum gypseum* in the present study. *Microsporum gypseum* was inhibited by chloroform extract in the concentration of 250 (µg/ml), whereas by ethylacetate extract in the concentration of 125 (µg/ml) and the aqueous extract in the concentration of 500 (µg/ml). Ethyl acetate extract was found to have higher activity than the chloroform and aqueous extract against both the organism *Microsporum canis* and *Microsporum gypseum* in the present study. Table 3 shows that *M.canis* was inhibited by Ethyl acetate extract of lemon grass leaves in the concentration of 62.5 (µg/ml), Wherease by Ethanol extract in the concentration of 250(µg/ml) and the aqueous extract in the concentration of 500(µg/ml). The *M.gypseum* was inhibited by ethyl acetate extract of lemon grass leaves in the concentration of 125(µg/ml), whereas by ethanol extract in the concentration of 250(µg/ml) and the aqueous extract in the concentration of 500(µg/ml).This study was correlated with the research. [22] In the present study, *Microsporum gypseum* was inhibited by ethyl acetate extract of combined activity of lemon grass and garlic bulb in the concentration of 62.5 (µg/ml). [23,] who proposed that *Allium sativum* was found to be most effective and also completely checking the mycelial growth of *Microsporum gypseum* at 10% concentration showing 83.09% inhibition in ethyl acetate extract. Least MIC and MFC recorded for combined ethyl acetate extracts of *Allium sativum* and *Cymbopogon citratus* extracts this shows the activity against pathogenic organism .[24] Analysis of sample *cymbopogan citratus* and *Allium sativum* and their constituents were identified with the help of GCMS. Seven compounds were identified with their retention time.citral(18.83),dimethyl sulfide(19.58)followed by nonadeconic acid(20.87), Brassidic acid(21.87), Behenic acid(22.82), hexodeconic acid(17.17)and Flavone(14.07) Fig 1. The antifungal activity presented by lemongrass and citral were similar and corrolates with literature, which indicated significant association between the effect and the presence of citral in lemongrass oil .[25] Literature points that citral acts as a fungicidal agent because it is able to form a charge transfer complex with an electron donor of fungal cells, resulting in fungal death. [26] Presence of dimethyl trisuifide showed the degradation of allicin. Allicin is formed in garlic when garlic bulb is crushed, enzyme allinase released which convert allinin into allicin.in this study the result showed that allicin got converted into disulfides and trisulfides.this work related with Gupta rainy et al whereas he identified four compounds with the help of GCMS which includes Diallylmonosulphide, Trisulfide methyl propenyl, Dimethylsulfide and Diallyl trisulfide. Table 6(a)&(b) The docking study between LAP1 and 2phenychromone-4-one shows binding energy --7.51kcal/mol, which has a interaction between the ALN 372 residue's O atom and the docking study between LAP1 and 1,1-dimethoxy-3,7-dimethylocta-2,6-diene shows binding energy -4.57 kcal/mol, which has four interactions between the ASN181 residue's O atom and O atom, GLY182 residue's N atom and O atom and ARG183 residue's N atom and O atom, SER184 residue's NE atom and O atom of1,1-dimethoxy-3,7-dimethylocta-2,6-diene. The docking score of all the compounds are low and these shows the above compounds are potent LPA1 inhibitor. Among the two compounds, 1,1-dimethoxy-3,7-dimethylocta-2,6-diene is a much potent inhibitor of LAP1 protein because it comes under Lipinski's rule of five and its docking score and interactions are also good between LAP1 and 1,1-dimethoxy-3,7-dimethylocta-2,6-diene. molecular docking confirmed in these studies. Table 7(a)&(b) The docking study between MEP1 and 2phenychromone-4-one shows binding energy --7.51kcal/mol, which has a four interaction between the ASN236 residue's N atom and O atom and VAL237 residue's ND2 atom and O atom, VAL237 residue's O atom and O atom, ASP239 residue's N atom and O atom.The docking study between MEP1 and 1,1-dimethoxy-3,7-dimethylocta-2,6-diene shows binding energy -4.57 kcal/mol, which has two interactions between the LEU40 residue's OD1 atom and O atom, GLY41 residue's N atom and O atom of1,1-dimethoxy-3,7-dimethylocta-2,6-diene.The docking score of all the compounds are low and these shows the above compounds are potent MEP1 inhibitor. Among the two compounds, 2phenychromone-4-one is a much potent inhibitor of MEP1 protein because it comes under Lipinski's rule of five and its docking score and interactions are also good between MEP1 and 2phenychromone-4-one. [27] molecular docking confirmed in these studies.

CONCLUSION

Molecular docking was performed between LAP1*(M.gypseum)*and ligand2phenychromone-4-one,1,1-dimethoxy-3, 7-dimethylocta-2, 6-diene. MEP1(*M.canis*) and 2phenychromone-4-one, 1, 1-dimethoxy-3, 7-dimethylocta-2, 6-diene from *Microsporum* sp. These proteins have good docking energy with ligands citral and flavones. The docking study reveals that hydrophobic interactions played a major role in ligand receptor interactions. Molecular docking have been applied in the identification of novel bioactive compounds.

CONFLICT OF INTEREST

Conflict of interest declared none

REFERENCES

1. Sharma M, Sharma M. Incidence of dermatophytes and other keratinophilic fungi in the schools and college playground soils of Jaipur, India. African Journal of Microbiology Research. 2010 Dec 18; 4 (24):2647-54.
2. Khanam AA. Isolation of keratinophilic fungi from soils of poultry farms. International Journal of Innovation in Engineering Research and Management ISSN 2348-4918, ISO 2000-9001 certified, E. 2014 Oct 10;1(5).
3. Gupta AK, Einarson TR, Summerbell RC, Shear NH. An overview of topical antifungal therapy in dermatomycoses. Drugs. 1998 May 1;55 (5):645-74.
4. Venugopal V. Antidermatophytic activity of neem (Azadirachta indica) leaves in vitro. Indian Journal of Pharmacology. 1994 Apr 1;26(2):141.
5. Treiber A, Pittermann W, Schuppe HC. Efficacy testing of antimycotic prophylactics in an animal model11Extracts presented at the 3rd Congress of the Deutsche Gesellschaft für Krankenhaushygiene (DGKH), 1996 (Stuttgart). International journal of hygiene and environmental health. 2001 Jan 1;204(4):239-43.
6. Ajello L. A new Microsporum and its occurrence in soil and on animals. Mycologia. 1959 Jan 1; 51 (1):69-76.
7. Araújo CR, Miranda KC, Fernandes OD, Soares AJ, Silva MD. In vitro susceptibility testing of dermatophytes isolated in Goiania, Brazil, against five antifungal agents by broth microdilution method. Revista do Instituto de Medicina Tropical de São Paulo. 2009 Feb;51 (1):9-12.
8. Aly AA, Hussein EM, Allam AD, Amein AM, El-Samawaty AM. Use of Trichoderma spp., Aspergillus spp., and Penicillium spp. to suppress damping-off of cotton seedlings. Journal of Agricultural Science Mansoura Univ. 2000; 25:7611-9.
9. Saadabi AM. Antifungal activity of some Saudi plants used in traditional medicine. Asian J Plant Sci. 2006;5 (5):907-.
10. Shelef LA, Jyothi EK, Bulgarellii MA. Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. Journal of Food Science. 1984 May 1; 49 (3):737-40.
11. Danlami U, Rebecca A, Machan DB, Asuquo TS. Comparative study on the Antimicrobial activities of the Ethanolic extracts of Lemon grass and Polyalthia Longifolia. Journal of Applied Pharmaceutical Science. 2011 Nov 11(9):174.
12. Geidam MA, Oyesola SA, Kokori AM. Effects of methanolic leaf extract of Adansonia digitata (Linn) on serum electrolyte levels in normal and alcohol fed rats. Pak J Biol Sci. 2004;7 (8):1404-6.
13. Gbile ZO. Ethnobotany, taxonomy and conservation of medicinal plants. The State of Medicinal Plants Research in Nigeria, Sofowora, A .(Ed.). University of Ibadan Press, Ibadan, Nigeria. 1986:13-29.
14. Y.Liu,L.Yang,Zu,Y.Zhang,and W.Wang "Development of an ILMS method for simultaneous extraction and distillation for determination for simultaneous extraction and distillation for determination of proanthocyanidins and essential oil in cortex cinnamoomi"journal of food chemistry, 2012; vol 135,pp2514-2512
15. Mondello L, Dugo P, Dugo G, Bartle KD. On-line HPLC—HRGC—MS for the Analysis of Natural Complex Mixtures. Journal of chromatographic science. 1996 Apr 1;34(4):174-81.
16. RNS Yadav andMunin agarwala."Phytochemical analysis of some medicinal plants".Journal of Phytology 2011 (3) ; 10-14
17. Lengauer T, Rarey M. Computational methods for biomolecular docking. Current opinion in structural biology. 1996 Jun 1; 6(3):402-6.
18. Rekha CB, Krishnaveni G. Incidence Of Microsporum Species From Different Soil And Water Samples From South Tamilnadu. Asian Journal of Multidisciplinary Studies. 2016 Feb 9; 4 (2).
19. Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells. Phytochemistry. 2005 Sep 30;6 6(17):2056-71.
20. Bonjar GS, Farrokhi PR. Anti-Bacillus activity of some plants used in traditional medicine of Iran. Nigerian Journal of Natural Products and Medicine. 2004;8(1):34-9.
21. Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamilnadu, India. Asian Pacific Journal of Tropical Disease. 2012 Aug 1;2(4):286-9.
22. Ellabib MS, Khalifa Z, Kavanagh K. Dermatophytes and other fungi associated with skin mycoses in Tripoli, Libya. Mycoses. 2002 Apr 1;45(3-4):101-4.
23. Cavallito CJ, Buck JS, Suter CM. Allicin, the antibacterial principle of Allium sativum. II. Determination of the chemical structure. Journal of the American Chemical Society. 1944 Nov; 66(11):1952-4.
24. Karmegam N, Karuppusamy S, Prakash M, Jayakumar M, Rajasekar K. Antibacterial potency and synergistic effect of certain plant extracts against food-borne diarrheagenic bacteria. International journal of biomedical and pharmaceutical sciences. 2008;2(2):88-93
25. Wannissorn B, Jarikasem S, Soontorntanasart T. Antifungal activity of lemon grass oil and lemon grass oil cream. Phytotherapy Research. 1996 Nov 1; 10(7):551-4.
26. Kurita N, Miyaji M, Kurane R, Takahara Y. Antifungal activity of components of essential oils. Agricultural and Biological Chemistry. 1981 Apr 1; 45(4):945-52.
27. Deepa .S, R. Deepika and S. Nandhini In-Silico Screening of Novel Urec Inhibitors from Eupatorium odoratum using Molecular Docking Study 2016*;* 173-176.

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