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Original Article

In vitro Antifungal Activity of Medicinal Plant Extracts Against Mucorales and Chemical Characterization of Garlic Extract by Gas Chromatography–Mass Spectrometry

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Abstract

The medicinal plant exhibits antimicrobial activities and therapeutic properties (antibacterial, antiviral, fungicidal, anti-cardiovascular disease, anticancer, antidiabetic, antihypertensive) over a wide range of microorganisms. This broad- spectrum was designed to evaluate the *in vitro* antimycotic activity of 5 medicinal plants against four selected fungal strains (*Rhizopus sp*., *Mucor sp*., *Absidia blakesleeana* and *Rhizomucor pusillus)*. In this research, the 20 plant extracts of 5 plant species were prepared using four solvents (petroleum ether, aqueous, ethanol and methanol) of required concentration by following maceration process. The antifungal activity of plants was determined by performing agar well diffusion method. Petroleum ether extract of *Allium sativum* showed more activity with zone of inhibition against *Rhizopus sp*. (15mm), *Mucor sp*. (25mm), *Absidia blakesleeana* (18mm) and *Rhizomucor pusillus* (19mm) as compared to other plants. Qualitative Phytochemicals analysis was screened of most potent antifungal plant, *Allium sativum*. Garlic petroleum ether solvent showed the presence of alkaloids, flavonoids, saponins and phenols. The minimum inhibitory concentration of most promising agent *Allium sativum* was examined by two-fold macro-dilution agar plate method against four fungal strains (*Rhizopus sp*. 0.67 mg/ml, *Mucor sp*. 10 mg/ml, *Absidia blakesleeana*1.25 mg/ml, and *Rhizomucor pusillus* 1.25 mg/ml).GC-MS analysis of petroleum ether extracts of *Allium sativum* (garlic) revealed various sulphur-containing compounds, including diallyl disulfide, diallyl trisulfide, and allyl methyl trisulfide, which are responsible for its antifungal activity. The major bioactive compounds present in the GC-MS analysis carried on petroleum ether extract of *Allium sativum* were 3-Vinyl-1,2- dithiacyclohex-5-ene (97.37% probability), 3-Vinyl-1,2- dithiacyclohex-5-ene (dithiins) (92.94%), 3-Vinyl-1,2- dithiacyclohex-5-ene (92.46%),1-Hexanone, 5-methyl-1- phenyl (86.8%), 3-Vinyl-1,2- dithiacyclohex-5-ene (86.20%). This work suggests that, following appropriate *in vivo* research, garlic extracts may be utilized as an antibiotic substitute.

***Keywords:** Antimycotic; Gas Chromatography–Mass Spectrometry (GC–MS); Mucormycosis; Medicinal Plants; Phytochemical Screening*

Introduction

A vast array of diseases occurs due to the fungal infections such as dermatophytosis, candidiasis, aspergillosis, and mucormycosis (Jangid & Begum, 2022). When the world was suffering from COVID-19 pandemic caused by SARS-CoV-2, looks towards in search of an effective drug or vaccines. In this the infected COVID-19 patients who were intaking steroids or having high blood sugar levels were triggered by black fungus also known as mucormycosis. Mucormycosis is a ubiquitous and the rmotolerant disease caused by a fungus of the class of Zygomycetes and the order of Mucorales (Mane & Wadikar, 2025). Mucormycosis cause infection in the sinuses (39%), lungs (24%), skin (19%), brain (9%), and gastrointestinal tract (7%), in the form of disseminated disease (6%), and in other sites (6%). It mainly causes by mucorales including *Mucor, Lichtheimia,*

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Syncephalastrum, Rhizomucor, Apophysomyces, Rhizopus and *Cunninghamella* species, which spread throughout the environment (decaying matter, soil, rotten wood, compost piles and leaves) (Hashem *et al.,* 2022). The combinations of clinical data and the isolation of fungus from clinical samples help to the probable diagnosis of mucormycosis. Rapid diagnosis, correction of predisposing factors, surgical resection, debridement and appropriate antifungal therapy are important to treatment of mucormycosis. It was hard and difficult to control infections due to imperfect diagnostic tools and therapeutic decisions. The mortality rate of mucormycosis is approximately 40% (Hashem *et al.,* 2022; Elsharawy *et al.,* 2024).

This fungal infection caused by the spread of the mucus in the nose by spores in the air. To date, only a few numbers of antifungal medications, such as polyenes, azoles, echinocandins, and flucytosine, are available in the market for the treatment of invasive fungal infections and has its own advantages and side effects (Lata *et al.,* 2023), so the plant-based products are getting more weightage, as they are safe to use, and comparatively easily available and cheap. Plants are also vital for human survival, providing food, health remedies, energy, and shelter (Anju & Kumar, 2024).

The plant products are an ample source of antimycotic drugs and antifungal activities further used for the treatment of various infectious diseases worldwide. Medicinal plants namely *Allium sativum, Catharanthus roseus*, *Cinnamomum verum*, *Azadirachta indica*, and *Syzygium cumini* have a long history in Ayurveda for treating many fungal infections but due to less studies present regarding the usage of these medicinal plants they are not in search (Aladejana *et al.,* 2024). Testing leaf extracts from medicinal plants for antimycotic activity may serve as a promising source for discovering novel antimicrobial agents. The aim of this research work was to determine antifungal activity of plant extracts against four fungal strains such as *Rhizopus sp.*, *Mucor sp.*, *Absidia blakesleeana* and *Rhizomucor pusillus* causing mucormycosis and to determine the minimum inhibitory concentration (MIC) and qualitative analysis of most potent plant extract.

One of the most effective and popular analytical methods for both qualitative and quantitative evaluation of volatile and semi-volatile substances found in medicinal plant extracts is gas chromatography–mass spectrometry (GC-MS) By fusing the molecular identification capacity of mass spectrometry with the high-resolution separation capability of gas chromatography, GC-MS enables accurate chemical detection, even at trace quantities. Researchers may create comprehensive phytochemical profiles using this method, which act as the chemical fingerprints of therapeutic plants (Patel *et al.,* 2021). Therefore, GC-MS is an essential tool in natural product research, helping to discover new compounds with potential pharmaceutical applications. Recent studies have shown that GC-MS analysis plays a crucial role in identifying antifungal and antibacterial agents from various plant species, supporting their use in treating microbial infections. GC-MS-based studies help to correlate specific chemical constituents with their biological functions, thus providing scientific validation to traditional medicinal claims (Kumar *et al.,* 2020).

Materials and Methods

Procurement of Fungal Cultures

Fungal cultures were collected from MTCC, Chandigarh (India). Each sample was tagged and placed in refrigerators at 4^0C in fresh PDA slants. All culture's name and MTCC No. used in the present study are shown in the Table 1.

Table 1: *Fungal Cultures Used in the Study*

Name of Cultures	**MTCC No.**
Rhizopus sp.	MTCC 6584
Mucor sp.	MTCC 157
Absidia blakesleeana	MTCC 918
Rhizomucor pusillus	MTCC 973

MTCC = Microbial Type Culture Collection

Preparation of Plant Extracts

Collection of Plants

The parts of medicinal plants were collected from different locations. The tap water was used to wash plant parts and then dried it to keep in 50˚C (oven) for 48 hrs and there after mixing to make powder (Kumar *et al.,* 2014). The list of plants used in this study are shown in Table 2 and Figure 1.

Table 2: *Medicinal Plant Parts Used*

Common Name	Botanical Name	Parts Used
Garlic	*Allium sativum*	Bulb
Chia seeds	*Salvia hispanica*	Seeds
Dalchini	*Cinnamomum verum*	Bark
Common lantana	*Lantana camara*	Flower
Neem	*Azadirachta indica*	Leaf

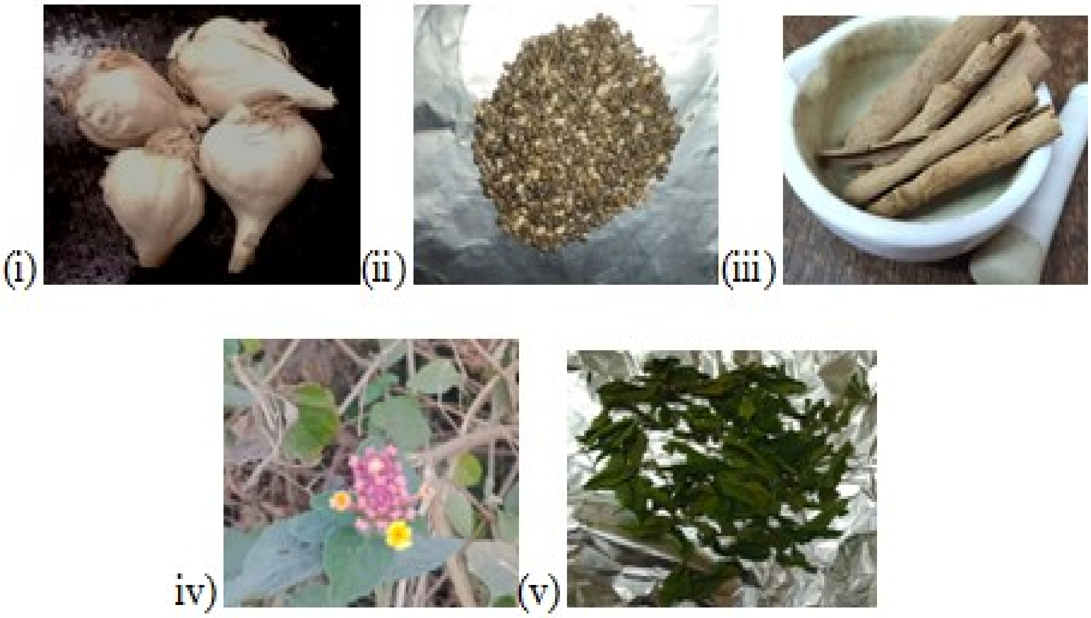

Figure 1: *Medicinal plants used in the present study (i)Allium sativum (bulb), (ii)Salvia hispanica (seeds), (iii) Cinnamomum verum (bark), (iv) Lantana camara (flower), (v)Azadirachta indica (leaf).*

Grinding, Soaking and Filtration Processes

Grinding the plant material with the use of mortar and pestle. After drying in the oven, powdered forms of 5 tested medicinal plants were obtained. Plant powder (40g) was dissolved and soaked in the respective solvent (ethanol 100%, methanol 100%, petroleum either 100% (AR Grade) and distilled water) to make the final volume 100ml and sealed with foil paper to prevent solvent evaporation. The mixture was incubated at room temperature for 24 hrs. After incubation, the extracts were filtered by sterilized filter paper of Whatman No.1. After filtration, the extract evaporation was done in water bath. Further, the extracts were evaluated for their antimycotic activity against fungi causing mucormycosis.

Inoculum Preparation

Fresh fungal cultures (3-4 days old) were used to prepare fungal inoculum suspension on potato dextrose agar plates. 5 ml of distilled sterile water was flooded on fungal culture plates. Tween 20 (5%) was added to facilitate the preparation of fungal strains. For fungal strains, rub the colonies with a sterile loop to prepare the fungal suspension/inoculums. The fungal culture was shaken for 15 seconds and transferred to a sterile tube. After filtering, the suspension was gathered in sterile tube. By removing most of the hyphae, this process created an inoculum that was mostly made up of spores (Faway *et al*., 2021).

Screening of Antifungal Activity of Medicinal Plant Extracts by Agar Well Diffusion Method

In this method, fungal inoculum was spread with sterile glass spreader. 6mm well was made with sterile borer. 100µL volume of the plant extract was poured in well of the inoculated agar plates. The plates were incubated for 3-5 days at 27˚C. The zone of inhibition on plates was observed and measured in mm (Hashem *et al*., 2022).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Most Promising Agent (Allium sativum)

The lowest concentration of an inhibitory agent needed to limit the growth after three to five days of incubation is known as the minimum inhibitory concentration (MIC) (Sharma *et al*., 2024). The lowest concentration of the antimicrobial agent required to kill the microorganism after additional incubation for five to seven days is known as the Minimal Fungicidal Concentration (MFC).

Five ml of standardized fungal suspension (10^6spores/ml) was mixed to the plant extracts after they have been combined with 5 ml of each extract at varying strengths (1 mg/ml to 0.125 mg/ml). 5ml of sterile PDB will have 1ml of extract and 1ml of fungus inoculum added before the tubes are placed in incubator at 25°C for 3-5 days. At regular intervals, it will be checked to see if any fungi are growing. The lowest concentration (maximum dilution) that exhibits no visible growth will be subject to additional observation and be deemed to be the Minimal Inhibitory Concentration (MIC). To find out whether the inhibition is temporary or permanent, a 100-microliter aliquot from tubes that are not growing will be sub-cultured on a PDA plate and placed in incubator at 25°C for a fungal colony.

Qualitative Analysis of Most Active Antimicrobial Metabolite

Qualitative screening of phytochemicals such as alkaloids, flavonoids, saponin, tannin, terpenoids and glycoside, was performed by the following method of Benayache *et al* (2017); Sarkar *et al* (2022).

Standard Deviation

The positive square root of mean of squared deviations from arithmetic mean, so called root means square deviation. The standard deviation is a measure of how widely values are dispersed from the average value (the mean).

STDEV uses the following formula: ∑ni=1(xi−¯x)^2.
Were,
x- Sum of all values of the variable
x- Mean of values
n- Sample size

Gas Chromatography-Mass Spectroscopy Profiling of Petroleum Ether Extract of Allium sativum

GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 8860) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD with Triple-Axis detector. The analysis method and the acquisition method were MS Default Method.pmx.: and different ramping 35-750 mzmethod.amx respectively. Helium gas was employed as the carrier gas and flowed at a column velocity of 1.0 millilitres per minute. The ion-source temperature is 230°C, the quad temperature is 150°C, the pressure is 7.0699 psi, the hold time is 1.3789 minutes, and the injector is 1 µl in split mode with a split ratio of 1:50 and the injection temperature is 300°C. Starting at 40°C for 5 minutes, the column temperature shifted to 150°V at a rate of 4°C per minute, with a temperature range of -60°C to 325°C (350°C). The Injection Draw Speed was 300 µL/min. A 6000 µL/min injection dispense speed was used. After increasing the temperature by 20 °C per minute to 250 °C, it was maintained for five minutes. Elution took a total of 35 minutes. Each component's relative percentage amount was determined by comparing its average peak area to the overall areas. To control the system and collect the data, the supplier's MS solution software was utilized.

Results

Antimycotic Microbial Activity of Plant Extracts Against Mucor Sp.

Of 20 extracts, *Allium sativum* petroleum either showed maximum zone of inhibition (25mm) followed by ethanolic extract (20mm), methanolic extract (18mm) and minimum in extract distilled water (16mm). *Azadirachta indica* Petroleum extracts showed antimycotic activity against with zone of inhibition of 13mm. No other extracts showed any inhibitory activity against *Mucor sp.* Clotrimazole (Control) showed antifungal activity against *Rhizopus* with zone of inhibition18mm (Table 3).

Table 3: *Antimycotic Activity of Medicinal Plant Extracts Against Mucor Sp.*

Medicinal Plants	**Antimycotic Activity of Medicinal Plant Extracts Against *Mucor sp.***			
	Methanol	**Ethanol**	**Petroleum ether**	**Distilled water**
Allium sativum	18mm ± 0.6	20mm± 0.8	25mm± 1.2	16mm± 0.7
Salvia hispanica	NA	NA	NA	NA
Cinnamomum verum	NA	NA	NA	NA
Lantana camara	NA	NA	NA	NA
Azadirachta indica	NA	NA	13mm± 0.5	NA
Clotrimazole (Control)	18mm			

Antimycotic Microbial Activity of Plant Extracts Against Rhizopus sp.

All the four *Allium sativum* extracts showed zone of inhibition ranging from 15mm to 18mm. *Salvia hispanica, Cinnamomum verum, Lantana camara* and *Azadirachta indica* extracts did not show antimycotic activity against *Rhizopus sp*. Clotrimazole (Control) showed antifungal activity against *Rhizopus* with zone of inhibition17mm. The results were shown in Table 4.

Table 4:*Antimycotic Activity of Plant Extracts Against Rhizopus Sp.*

Medicinal Plants	**Antimycotic Microbial Activity of Plant Extracts Against Rhizopus sp.**			
	Methanol	**Ethanol**	**Petroleum ether**	**Distilled water**
Allium sativum	16mm± 0.6	18mm± 0.7	15mm± 0.6	17mm± 0.6
Salvia hispanica	NA	NA	NA	NA
Cinnamomum verum	NA	NA	NA	NA
Lantana camara	NA	NA	NA	NA
Azadirachta indica	NA	NA	NA	NA
Clotrimazole (Control)	17mm			

Antimycotic Microbial Activity of Plant Extracts Against Absidia blakesleeana

Out of 5 medicinal plants, only *Allium sativum* extracts (ethanol, petroleum ether and distilled water) showed antimycotic activity against *Absidia blakesleeana* (ethanol with 15mm zone of inhibition, petroleum ether (18mm) and distilled water (14mm). Clotrimazole (Control) showed antifungal activity against *Absidia blakesleeana* with zone of inhibition 22mm. None of the activity shown by remaining 4 plants in either of the solvents (Table 5).

Table 5: Antimycotic Activity of Plant Extracts Against *Absidia blakesleeana*

Medicinal Plants	**Antimycotic Activity of Plant Extracts Against *Absidia blakesleeana***			
	Methanol	**Ethanol**	**Petroleum ether**	**Distilled water**
Allium sativum	NA	15mm± 0.6	18mm± 0.7	14mm± 0.5
Salvia hispanica	NA	NA	NA	NA
Cinnamomum verum	NA	NA	NA	NA
Lantana camara	NA	NA	NA	NA
Azadirachta indica	NA	NA	NA	NA
Clotrimazole (Control)	22mm			

Antimycotic Microbial Activity of Plant Extracts Against Rhizomucor Pusillus

Out 5 medicinal plants, *Allium sativum* ethanolic and petroleum ether extracts showed the activity against *Rhizomucor pusillus* with zone of inhibition (13mm) and petroleum ether (19mm). None of the activity shown by remaining 4 plants in either of the solvents. Clotrimazole (Control) showed antifungal activity against *Rhizomucor pusillus* with zone of inhibition 30mm (Table 6)**.**

Table 6: Antimycotic Activity of Plant Extracts Against *Rhizomucor pusillus*

Medicinal Plants	**Antimycotic Activity of Plant Extracts Against *Rhizomucor pusillus***			
	Methanol	**Ethanol**	**Petroleum ether**	**Distilled water**
Allium sativum	NA	13mm± 0.5	19mm± 0.7	NA
Salvia hispanica	NA	NA	NA	NA
Cinnamomum verum	NA	NA	NA	NA
Lantana camara	NA	NA	NA	NA
Azadirachta indica	NA	NA	NA	NA
Clotrimazole (Control)	30mm			

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Most Promising Agents

Based on maximum zone of inhibition, *Allium sativum* (bulb) petroleum ether extract was selected as most potent antifungal agent to determine minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The MIC and MFC were determined by using macro-dilution agar plate techniques of most promising agent (*Allium sativum* (bulb) with petroleum ether extract). The petroleum ether *Allium sativum* (bulb) extract showed minimum inhibitory concentration (MIC) of 10.0 mg/ml against Mucor sp., 0.67 mg/ml against Rhizopus sp., 0.67 mg/ml against *Rhizomucor pusillus* and 1.25 mg/ml against *Absidia blakesleeana* and minimum fungicidal concentration (MFC) of 20.0 mg/ml against Mucor sp., 1.25 mg/ml against Rhizopus sp., 0.67 mg/ml against *Rhizomucor pusillus* and 2.5 mg/ml against *Absidia blakesleeana*. The results of MIC and MFC are presented in Table 7.

Table 7: *Minimum Inhibitory Concentration and Minimum Fungicidal Concentration against All Four Fungal Strains on Different Concentrations*

Concentration of Extracts (Mg/Ml)								
Pure fungal cultures	20	10	05	2.5	1.25	0.67	MIC	MFC
Mucor sp.	+	-	-	-	-	-	10	20
Rhizopus sp.	+	+	+	+	+	-	0.67	1.25
Rhizomucor pusillus	+	+	+	+	+	-	0.67	0.67
Absidia blakesleeana	+	+	+	+	+	-	1.25	2.5

Qualitative Analysis of Most Active Antifungal Extract

Phytochemical screening of most potent active antifungal extract, *Allium sativum* was done. *Allium sativum* petroleum ether showed the presence of alkaloids, flavonoids, saponins and phenols. *Allium sativum* distilled water showed the presence of alkaloid and saponin and absence of all the other tested phytochemicals (Table 8).

Table 8:*Qualitative Screening of Active Antimicrobial Metabolite Present in Most Potent Plant Allium sativum* (Garlic)

Qualitative Phytochemical Analysis of *Allium sativum*				
Solvents Phytochemicals	**Ethanol**	**Methanol**	**Distilled Water**	**Petroleum Ether**
Alkaloids	-	-	+	+
Flavonoids	-	-	-	+
Saponins	-	-	+	+
Tanins	-	-	-	-
Glycosides	-	-	-	-
Phenols	-	-	-	+

Gas Chromatography-Mass Spectroscopy Profiling of Petroleum Ether Extract of Allium sativum

The GC-MS examination of the *Allium sativum* petroleum extract identified many compounds, with retention times ranging from 3.666 to 17.626 minutes. The list of major compounds was depicted from the GC-MS analysis of petroleum extracts of *Allium sativum*. The chromatogram was presented in Fig 2, while the chemical constituents with their retention time (RT), molecular formula, Cas Number, Score, Rev. Score and Probability % are presented in Table 9. The following bioactive compounds were present in the GC-MS analysis carried on petroleum ether extract of *Allium sativum*: 3-Vinyl-1,2-

dithiacyclohex-5-ene (97.37% probability), 3-Vinyl-1,2- dithiacyclohex-5-ene (dithiins) (92.94%), 3-Vinyl-1,2- dithiacyclohex-5-ene (92.46%),1-Hexanone, 5-methyl-1- phenyl (86.8%), 3-Vinyl-1,2-dithiacyclohex-5-ene (86.20%), Ethanol, 2-(2- propynyloxy)- (71.25%), 1,2,4-Triazol-1-ylacetic acid (71.42%), Cyclohexane, methyl-(69.96%), 3-Vinyl-1,2- dithiacyclohex-4-ene (62.74%), Cis-1-methyl-3-n- nonylcyclohexane (62.72%), Heptane, 2-methyl- (59.49%), Cyclohexane, ethyl- (58.25), Acetamide, 2-cyano- (55.82), Hexane, 2,4-dimethyl- (53.4%), 2-Ethylthiolane, S,S- dioxide (48.42%), 3-Vinyl-1,2- dithiacyclohex-5-ene (47.96), Cyclopentane, 1,2,4-trimethyl-, (1α,2β,4α)- (46.53%), 1,3-Propanediamine (46.91%), Cyclopentane, 1,2,3-trimethyl-(38.33 %), Cyclohexane, 1,1,3- trimethyl-(36.82), Benzene, 1,3-dimethyl-(36.5%), Ethylbenzene (35.22%), 2,6-Pyrazinediamine (33.57%), Octane, 3-methyl-(32.81%), Benzene, 1,3-dimethyl-(32.58%), Octane, 3-methyl-(32.46%), Mesitylene (32.19%), Benzene, 1,3-dimethyl- (31.36%), Cyclohexane, propyl-(30.83%), Cis-1-methyl-3-n-nonylcyclohexane (30.41%), Cyclopropane, 1-butyl-2- (2-methylpropyl)-(30.22%), Butane, 1-(ethenyloxy)-3-methyl- (30.07%),. As a result of the presence of these important components, the petroleum extract of *A. sativum* could have an important therapeutic significance.

Table 9: *GC-MS Peak Report for Total Ion Chromatogram (TIC) of Phytocompounds Present in the Petroleum Ether Extract of Allium sativum*

Peak	Retention Time (RT)	Chemical formula	Cas #	Score	Rev. Score	Probability %
1	3.666	1,3- Propanediamine	109-76-2	752	752	46.91
2	3.722	Cyclohexane, methyl-	108-87-2	932	932	69.96
3	3.925	Cyclopentane, ethyl-	1640-89-7	895	895	62.85
4	4.534	Heptane, 2-methyl-	592-27-8	878	878	59.49
5	5.05	2-Ethylthiolane, S,S- dioxide	10178-59-3	809	809	48.42
6	5.329	Hexane, 2,4-dimethyl-	589-43-5	923	925	53.4
7	6.183	Cyclohexane, ethyl-	1678-91-7	902	908	58.25
8	9.611	Ethanol, 2-(2- propynyloxy)-	3973-18-0	625	625	71.25
9	10.408	Cis-1-methyl-3-n-nonylcyclohexane	39762-39-5	757	795	62.72
10	10.557	1-Hexanone, 5-methyl-1- phenyl	25552-17-4	640	640	86.8
11	12.155	1,2,4-Triazol-1-ylacetic acid	28711-29-7	770		71.42
12	13.649	Acetamide, 2-cyano-	107-91-5	639	771	55.82
13	16.874	3-Vinyl-1,2- dithiacyclohex-4-ene	62488-52-2	648	648	62.74
14	16.885	3-Vinyl-1,2- dithiacyclohex-5-ene	62488-53-3	654	722	47.96
15	17.597	3-Vinyl-1,2- dithiacyclohex-5-ene	62488-53-3	838	883	92.94
16	17.608	3-Vinyl-1,2- dithiacyclohex-5-ene	62488-53-3	823	859	86.20
17	17.617	3-Vinyl-1,2- dithiacyclohex-5-ene	62488-53-3	835	872	92.46
18	17.626	3-Vinyl-1,2- dithiacyclohex-5-ene	62488-53-3	804	858	97.37

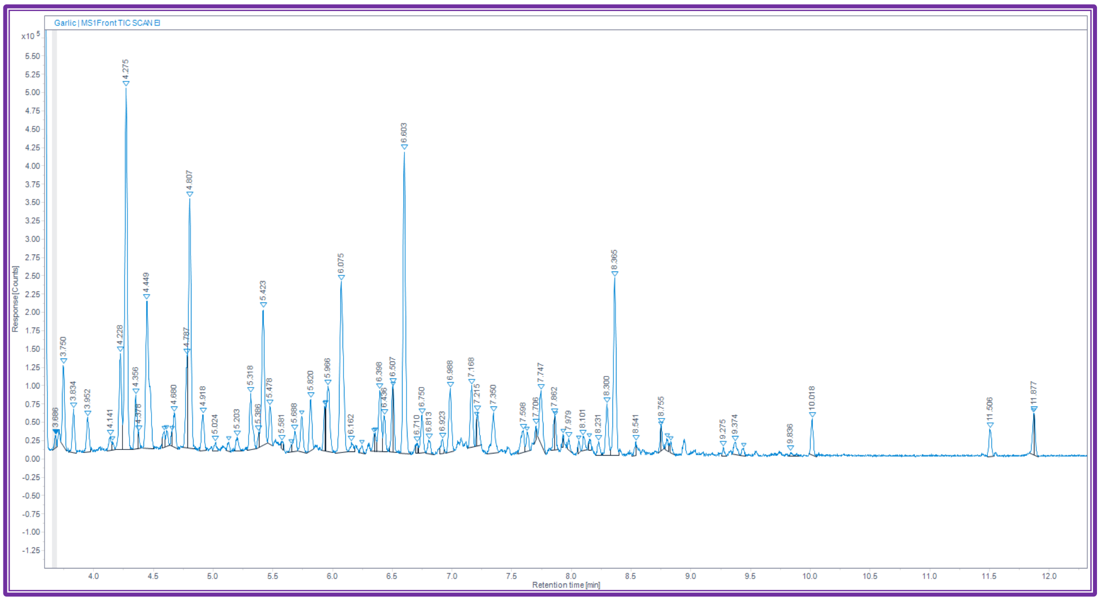

Figure 2: *Total Ion Chromatogram (TIC) of Petroleum Ether Extract of Allium Sativum*

Discussion

Very few currently available antifungal agents are being used to treat fungal infections caused Mucormycosis. In the present study, *Allium sativum* showed maximum inhibition in petroleum, ether extract followed by ethanolic extract, methanolic extract and minimum in extract distilled water (16mm). *Allium sativum* plants have been known for their antimycotic properties since ancient times. Recent studies have shown that the leaves and bulbs of these plants are rich in phenols, flavonoids and thiosulfinates, compounds that have proven antimicrobial, antioxidant or antitumor activity (Stupar *et al.,* 2022). Naturally, in *Allium* bulbs or leaves allicin is formed after crushing or cutting plant organs under the action of the alliinase enzyme (Choudhary *et al.,* 2022; Barbu *et al.,* 2023).

The study revealed that the inhibitory effect of *Allium sativum* aqueous extract against *Rhizopus sp.* was found to be average of inhibition shown 20mm by using the method disc diffusion method (Ghajiri, *et al.,* 2023; Barbu *et al.,* 2023). According to Nimoshini *et al.,* (2023) antimicrobial activities of garlic against *Mucor sp.* observed that though inhibitory effect of the aqueous extracts found to be in range of 40mm by using the well diffusion assay it may be due to the different constituents present in them. The antifungal activity of water and alcoholic extracts from black pepper on the growth of some fungi isolated from food products are checked against *Rhizopus sp.* The outcomes showed that sensitivity of the fungi varied according to the type of extract, with the alcoholic extract being more efficient contrasted to water extracts (Mohsen *et al.,* 2024).

The antifungal activity of *Salvia hispanica* was observed by using the disc diffusion method against *Rhizopus sp*. (Rahmoune *et al.,* 2024). In our study no activity was observed against tested fungal strains. It is due to the different methods used or different species used in the research. The antifungal activity of aqueous and ethanolic garlic extract was determined on some selected fungi namely, *Fusarium* sp. and *Rhizopus sp.* From the results it was clear that ethanol extract showed more activity when compared to aqueous extract (Kutawa *et al.,* 2018). *Azadirachta indica* petroleum extracts showed antimycotic activity against with zone of inhibition of 12mm and 13mm respectively. No other extracts showed any inhibitory activity against *Mucor sp.* (Table 4). The activity may be due to presence of phytochemicals including alkaloids, carbohydrates, flavonoids, resins, steroids, tannins, inorganic acids, organic acids, phenolic compounds, amino acids, protein and coumarins (Arasu *et al.,* 2021). All the four *Allium sativum* extracts showed zone of inhibition ranging from 15mm to 18mm activity against *Rhizopus sp*. *Salvia hispanica, Cinnamomum verum, Lantana camara* and *Azadirachta indica* extracts did not show antimycotic activity against *Rhizopus sp*.

Lantana camara (leaf) extracts (aqueous and ethanol) were screened for antifungal activity which shows the zone of inhibition of 18mm and 21mm respectively against *Mucor* sp. (Jangid & Begum, 2022). According to present study no activity was observed in either of the solvents, which may be due to the plant part used in individual research, and difference in method of extraction.

In this study, only *Allium sativum* extracts (ethanol, petroleum ether and distilled water) showed antimycotic activity against *Absidia blakesleeana* (ethanol with 15mm zone of inhibition, petroleum ether (18mm) and distilled water (14mm). No more literature available on antifungal activity of plant extracts against *Absidia blakesleeana.* A study done by Sharma *et al.,* (2024), *A. blakesleeana* fungus was controlled by antidiabetic medicinal plants, *F. religiosa, C. roseus, O. Tenuiflorum, T. arjuna, M. alba, and S. cumini* by disc diffusion method. *F. religiosa* showed maximum antimycotic activity with zone of inhibition of 32.5 mm followed by *Morus* spp. (20.5 mm), *Catharanthus* spp. and *Ocimum* spp. (20 mm), *Syzgium spp*. (19.5 mm) and minimum zone of inhibition in *Terminalia spp*. (14 mm). Out of 5 medicinal plants, *Allium sativum* ethanolic and petroleum ether showed the activity against *Rhizomucor pusillus* with zone of inhibition (13mm) and petroleum ether (19mm) None of the activity shown by remaining plants in either of the solvents.

In the present investigation, the petroleum ether *Allium sativum* (bulb) extract showed minimum inhibitory concentration (MIC) of 10.0 mg/ml against *Mucor sp.*, 0.67 mg/ml against *Rhizopus sp.*, 0.67 mg/ml against *Rhizomucor pusillus* and 1.25 mg/ml against *Absidia blakesleeana* and minimum fungicidal concentration (MFC) of 20.0 mg/ml against *Mucor sp.*, 1.25 mg/ml against *Rhizopus sp.*, 0.67 mg/ml against *Rhizomucor pusillus* and 2.5 mg/ml against *Absidia blakesleeana*. The results of MIC and MFC are presented in Table 8 and figure 10.The garlic extract contained thiosulfinate, flavonoids and polyphenols as major compounds and demonstrated the highest efficiency against the *Aspergillus versicolor* (MIC 3.12–6.25 mg/ml)*, A. ochraceus* (MIC: 3.12 mg/mL)*, Penicillium expansum* (MIC 6.25–12.5 mg/ml), and *A. niger* (MIC 3.12–50 mg/ml) strains (Corbu *et al.,* 2021).

Clotrimazole used as positive control showed maximum antifungal activity against *Rhizomucor pusillus* with zone of inhibition 30mm followed by *Rhizomucor pusillus* (22mm)*, Mucor sp.* (18mm) and minimum in *Rhizopus* (17mm). The first-line treatment for this life-threatening fungal infection is liposomal AmB. Due to residuals concerns, isavuconazole and new posaconazole formulations have been recommended for clinical use, but only as a second-line treatment after liposomal AmB (Brunet &Rammaert, 2020; Hussain *et al.,* 2023). *Allium sativum* Petroleum ether extract showed 25mm zone of Inhibition against *Mucor* sp. In comparison with pure form of Clotrimazole showed 17 mm zone of Inhibition. This may be due to the combination effect of secondary metabolites such as alkaloids, flavonoids, saponins and phenols in crude extract (*Subramanian et al., 2020).*

In current study, *Allium sativum* petroleum ether showed the presence of alkaloids, flavonoids, saponins and phenols. *Allium sativum* distilled water showed the presence of alkaloid and saponin and absence of all the other tested phytochemicals in ethanolic and methanolic extracts. Hu *et al* (2024) analysed phytochemical constituents of petroleum ether extract of *Allium sativum* and concluded the presence of alkaloid and organic sulphur compounds. Also, the herbal medicinal plants *Allium sativum* contain several active phytoconstituents such as alkaloids, amino acids or primary and secondary amine, anthraquinones, flavonoids, glycosides, phlorotannins, tannins, saponins, and terpenoids present in the ethanolic extract (Panda *et al.,* 2024).

A large variety of volatile chemicals can be identified and quantified using GC-MS analysis based on retention times (RT), areas, and area sum (%). Since it indicates how long it takes for a single molecule to exit the chromatographic column, RT is a crucial component of compound identification. It is also a crucial factor in identifying components using molecular differences. Individual compound signal intensities are represented by the area values, and each value is directly correlated with the compound's concentration in the extract sample. The area sum percentages correspond to the type of relative proportions of each compound to the total chemical composition of Extract (Faqer *et al*., 2024; Tran *et al*., 2024; Wirtu *et al*., 2024). GC-MS analysis results were similar with Chen *et al* (2018) as GC–MS revealed that the major active ingredients were 3-vinyl-1,2-dithiacyclohex-5-ene and 3-vinyl-1,2-dithiacyclohex-4-ene in *Allium sativum*. The broad-spectrum antifungal activity may be due to these two major compounds. Chen *et al* (2018) observed that the antimicrobial activity of garlic extracts may be due to destruction of the structural integrity of cell membranes, leading to cell death.

The major bioactive compounds were present in the GC-MS analysis carried on petroleum ether extract of *Allium sativum* were 3-Vinyl-1,2- dithiacyclohex-5-ene (97.37% probability), 3-Vinyl-1,2-dithiacyclohex-5-ene (dithiins) (92.94%), 3-Vinyl-1,2- dithiacyclohex-5-ene (92.46%). The primary compound is Allyl trisulfide (41.29 %), while Diallyl disulfide (16.73 %) and 2-Vinyl-1,3-dithi-4-ene (19.88 %) composed a large part of the chemical composition as well (Kobacy *et al.,* 2025). The GC-MS analysis of Garlic essential oil (GEO) revealed that, Allyl methyl trisulfide (13.10%), Di-allyl sulphide (9.47%) and Di-allyl tetrasulfide (4.38%) were the major components which indicates the presence of broad-spectrum antimicrobial constituents in garlic essential oil (Ashraf *et al.,* 2019). According to Hu *et al* (2024) the Petroleum Ether Extract of *Allium sativum* (aerial parts) contains abundant plant sterols (phytosterols), such as 9,19-Cyclolanost-24-en-3-ol, (3. beta.)- (Cycloartenol), 9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)- and gamma-sitosterol. Hu *et al* (2024) also observed that these compounds significantly reduce the contents of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), alanine transaminase (ALT) and aspartate transaminase (AST) in serum of hyper lipidemic mice and increase the contents of high-density lipoprotein cholesterol (HDL-C). They could enhance the activity of superoxide dismutase (SOD) in liver and reduce the level of malondialdehyde (MDA).

GC-MS analysis of petroleum ether extracts of *Allium sativum* (garlic) reveals various sulphur-containing compounds, including diallyl disulfide, diallyl trisulfide, and allyl methyl trisulfide, which are responsible for its antifungal activity. Garlic extracts showed a broad-spectrum fungicidal effect against a wide range of fungi including *Candida, Torulopsis, Trichophyton, Cryptococcus, Aspergillus*, *Trichosporon,* and *Rhodotorula* species. Recently, garlic extract was found to inhibit the *Meyerozymaguilliermondii* and *Rhodotorulamucilaginosa* germination and growth (Pârvu *et al.,* 2019). Another study reported the antifungal activity of various *A. sativum* extracts namely aqueous, ethanolic, methanolic, and petroleum ether against human pathogenic fungi such are *Trichophyton verrucosum*, *T. mentagrophytes*, *T.rubrum*, *Botrytis cinerea*, *Candida* species, *Epidermophyton floccosum*, *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifera, Microsporumgypseum, M. audouinii*, *Alternaria alternate, Neofabraea alba,* and *Penicillium expansum* (Fufa, 2019; Batiha *et al.,* 2020). Bioactive compounds may cause irreversible ultrastructural changes in the fungal cells, loss of structural integrity and affect the germination ability as mechanism of action. Several garlic extract and its bioactive compounds have been reported for anti-tumour activity with various cancer/tumour cell lines (Antony *et al.,* 2019).

Limitations

The present study was based only on *in vitro* testing against limited number of strains, which do not fully replicate the complexity of a living system. The in vitro efficacy of tested plant extracts against fungi causing mucormycosis in human or animal models may differ due to host immunity, metabolism, and bioavailability.

Future Scope

For future prospects, research should focus on *in vivo* study of active antifungal extracts of *Allium sativum* extracts against fungi causing mucormycosis along with isolation, purification and characterization of active compounds, and detailed mechanistic investigations. Additionally, advanced techniques with formulation development and toxicity assessment can help translate these findings into effective clinical applications.

Conclusion

The present study demonstrates that *Allium sativum* (garlic) petroleum ether extract showed the best antimycotic activity against all tested fungi followed by *Azadirachta indica* extracts. The major findings include *Allium sativum* petroleum ether extract showed better zone of Inhibition against *Mucor* sp. as compared with Clotrimazole. Phytochemical screening of the garlic petroleum ether extract revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, and phenolic compounds, which are likely responsible for the observed antifungal activity. The findings of this study may need further purification and characterization along with *in vivo* study and validation with larger sample size before use for commercial purposes. Considering the global rise in antifungal resistance, plant-derived therapeutics may serve as a promising alternative or complementary strategy for the management of fungal infections. The current findings highlight the need for future research focusing on isolation of active molecules, structural elucidation, and *in vivo* antifungal studies to validate the efficacy and safety of these plant-based antifungal agents.

Conflict of Interest

The authors declare that they have no competing interests.

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