Health Biotechnology and Biopharma (2023), 7(4): 24-43

Original Research Article

GC/MS analysis and antifungal activity of M. Chamomilla extracts against molecularly identified keratinophilic fungi

Afrah Talib Abdullah [1], Kadhim Fadhil Kadhim [1,*]
[1]Department of Biology, College of Education, Qurna, University of Basrah, Iraq

***Corresponding author:** Kadhim Fadhil Kadhim, Department of Biology, College of Education, Qurna, University of Basrah, Iraq, E-mail: kadhim.fadhil@uobasrah.edu.iq*
DOI: 10.22034/HBB.2024.02

Received: November 15, 2023; **Accepted:** December 23, 2023

ABSTRACT

The objective of this study was to isolate and genetically identify keratinophilic fungi associated with humans, test their susceptibility to nystatin, and evaluate the effectiveness of Methanol Extract (ME) from chamomile flowers against these fungi. No cytotoxic effects were observed in the extract cytotoxicity assay using human blood solution. The antifungal activity of the chamomile extract varied among different fungal isolates and extract concentrations. The extract exhibited a strong inhibitory effect on fungal growth, depending on the type of fungal strain and concentration. Statistical analysis revealed significant differences between the effects of the extract and the conventional antifungal medication (nystatin) on specific fungi. GC/MS analysis of the chamomile extract identified several bioactive compounds, including flavonoids, phenolic compounds, and essential oils. The presence of these bioactive components in the extract likely contributes to its high antifungal activity against the tested fungal infections.

***Keywords*:** GC/MS, nystatin, keratinophilic fungi

INTRODUCTION

The skin is the largest organ in the body, a haven for microbiota, and the first line of defense against noxious substances from the environment and pathogenic bacteria. In addition to serving as a physical barrier, the skin's resident immune system also forms a dynamic system that is essential for preventing infections, repairing damage, and preserving tissue homeostasis. Dermatophytoses (ringworms), which are

among the most common skin infections in humans, are believed to have a global incidence of 20 to 25 percent in the healthy population [1-3]. These infections are brought on by filamentous fungi, which were once keratin-digesting creatures of the soil but have since evolved to live as parasitic microbes on both animals and people. Therefore, subacute or chronic infections with varying degrees of inflammation in immunocompetent persons are caused by hyphae superficial penetration into the skin, hair, and nails in dermatophytoses. Dermatophytes are divided into five genera according to recent taxonomic changes: Microsporum, Arthroderma, Trichophyton, Epidermophyton, and Nannizzia [4].

Additionally, different species within them have been classified as geophilic, zoophilic, and anthropophilic fungi according to their adaptation to particular ecological niches and hosts. Trichophyton rubrum and Epidermophyton floccosum are two anthropophilic species that are well adapted to people and frequently cause chronic infections with no clinical signs. Contrarily, dermatophytes from animals or soil (Nannizzia gypsea/Microsporum gypseum) are frequently isolated from patients with mild to highly inflammatory dermatophytosis but with lesions that are prone to spontaneous resolution [4]. These dermatophytes include Microsporum canis, Trichophyton/Arthroderma benhamiae, Trichophyton mentagrophytes, etc [5].

Contrarily, individuals who are immunosuppressed, particularly those who have cell-mediated immunity deficiencies due to conditions like HIV/AIDS, transplant, neoplasia, diabetes, or corticosteroid therapy, are more vulnerable to these infections and frequently develop extensive superficial lesions that do not respond to conventional antifungal medication [6]. A considerable rise in treatment-recalcitrant, recurring, and chronic dermatophytosis was recently noted in India, perhaps as a result of the careless use of antibiotic and corticosteroid medication combinations [7].

The prevalence of serious fungal infections caused by Candida spp. has increased worldwide in recent years, particularly in immunocompromised individuals [8], and these infections can present superficially or cause serious systemic disease [9]. The limited number of antifungal classes [10], the rise in serious infections caused by Candida spp [11], and the rise in microbial resistance to drugs [12] frequently as a

result of repeated or long-term therapies [13] are driving forces in this trend. It has been noted that innovative alternative treatments for these fungi infections are urgently needed[14]. Compared to chemical and synthetic pharmaceuticals, natural goods are preferred as biocompatible and non-toxic drugs without side effects in medicine. Natural extracts with biological and therapeutic qualities that are given to humans and animals as dietary ingredients or in particular pharmaceutical preparations have drawn more attention in recent years. Promising treatments for various ailments are thought to be derived from medicinal plants. Many plants' biological and pharmacological characteristics, however, remain unknown [15].

Recent research suggests that plants are natural sources of new antibacterial substances with a wide range of medicinal applications [14]. The efficiency of plant extracts against microorganisms has been demonstrated in numerous research [16].

The purpose of this study was to isolate, genetically identify, assay the susceptibility of keratinophyllic fungi connected to humans to Nystatin (NY), and assess the effectiveness of Methanol Extracts (ME) from Matricaria chamomilla (chamomile) flowers against the isolated fungi. Additionally, investigate the ME chemical composition using Gas Chromatography/Mass Spectrometry (GC/MS) analysis to identify active chemicals.

MATERIALS AND METHODS

Ethical Approve

This research was registered in University of Basrah, College of Education/ Qurna. Human ethical approval was obtained from Iraqi Ministry of Health, Directorate of Health in Basra in their book No. 724 on September 19, 2019. The research did not violate the patient's rights or privacy, nor did the research address personal information such as patient pictures, etc. Informed consent was obtained from each patient included in the study

Sampling, Isolation and DNA extraction

Twenty samples were taken from the skin, hair, and nails of people who had fungal infections. Dermatologist Dr. Laith Globe Lazim Al-Kinani (Master of Dermatology, PhD in Dermatology, Iraqi Ministry of Health, Al-Fayhaa Teaching Hospital) aided in the diagnosis of clinical cases. The samples were delivered to the clinical

mycology laboratory at the College of Sciences at the University of Basrah.

Using a sterile scalpel, samples were taken from patients and kept in sterile plastic bags until they were cultured on Sabouraud Dextrose Agar (SDA), HiMedia Co., India, and incubated at 37 °C for seven to thirty days to identify the samples molecularly and determine their susceptibility to Nystatin and other natural products. The genomic DNA for the 12 positive samples was extracted using Al-Mahmoud [17] methodology. The DNA samples were kept at -80 °C pending PCR amplification after electrophoresis in 0.5 % agarose gel stained with ethidium bromide (Biotech Co, Canada).

Polymerase Chain Reaction (PCR) product

Two universal primers, ITS1(F-5-TCC GTA GGT GAA CCT GCG G-3) and ITS4(R-5-TCC TCC GCT TAT TGA TAT GC-3), were used in the PCR created by White et al. [18] to amplify the Internal Transcribed Spacer region (ITS 1-5.8S-ITS2). The PCR Master Mix (5 µl, Bioneer Co, Korea), DNA template (5 µl, Bioneer Co, Korea), and Nuclease Free Water (25 µl, Bioneer Co, Korea) were combined to create the DNA amplification solution. DNA fragments were separated in a 2 % agarose gel, and the sizes of the products were compared with molecular markers (100–2000 bp, Bioneer Co., Korea). The fragments were then inspected with a UV transilluminator and compared to ladder fragments.

PCR Product Sequencing of ITS1- 5.8S – ITS2 rDNA region

Macrogen Co, South Korea, sent the PCR products for sequencing. The NCBIs "BLAST" was used to identify the PCR sequencing products of fungi.

Creation of M. Chamomilla Methanol Extract (ME)

Weighed and ground into a powder, dry chamomile flowers were used to make a 5 % w/v suspension in a flask by adding methanol alcohol. The temperature was then held at 37 °C for 4 hours while the flask was shaken at 200 rpm. After shaking, the suspension was run through a series of Whatman Filter Papers before the flask was cooled to ambient temperature run through a 0.22 micron filter at the end. According to Srivastava and Gupta [19], the filtered ME was dried at ambient temperature and kept at -20 °C until use.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

One mL of ME was taken for GCMS/MS (Shimadzu Co GCMS-QP2010 Ultra, Japan) analysis. GC-MS analysis was carried out at the Basra oil company Laboratory, by using an Agilent Technologies , 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI Signal detector , using HP-5ms 5 % phenyl , 95 % methyl siloxane (30m*250um*0.25) , the oven temperature was set at 40 °C hold for 5 min then raised to 8 °C/min to 300 °C for 20 min , Helium carrier gas flow rate was 1 ml/min and purge flow 0f 3 ml/min .The injection mode was pulsed Splitless with injection temperature 290 °C and the injection sample volume was 0.5 micro letter. The mass spectrometer used Ion Source Temperature 230 °C , With scan speed 1562(N2) , and the mass range 44-750 m/z , Data was run through the NIST 2014 ,2020 Library data base as an additional tool to confirm identity of compounds. The name, Molecular Weight (MW), Retention Time (RT), peak area and structure of the active constituents of ME we ascertained.

Cytotoxicity test

The produced ME underwent a biocompatibility test against human fresh blood using the Nair et al. method [20].

Evaluation of the efficacy of ME and antifungals against isolated fungi

A portion of the colony was taken and mixed with 5 ml of physiological saline solution (normal saline), which was placed on the dishes until dry. The mixture was then added to the extract in wells that had been prepared with a diameter of 6 mm, as it was filled with 50 microliters. This method, known as the agar well diffusion method [21], was used to study the sensitivity of ME. Based on the same steps above and the same concentrations, the antifungal (Nystatin, Sapha Co, Iraq) was tested on isolated keratinophilic fungi.

Statistical description

Data were analysis by the ANOVA test at significant level 0.05.

RESULTS

Molecular Identification

The molecular results of sequencing the ITS region revealed the presence of multiple fungal species among the isolates. The identified species included Scopulariopsis cordiae, Scopulariopsis stercoraria, Scupulariopsis brevicaulis, Trichophyton rubrum, Trichophyton mentagrophytes, and Microsporum canis. Among these species, our research showed that Scopulariopsis brevicaulis was the

most prevalent (Table 1, Figure 1). Using this method, we were able to register four new keratinocyte fungal isolates in DNA Data Bank of Japan (DDBJ) for the first time ever, as fungi had certain point mutations that were found in the sequence data.

Cytotoxicity

Various amounts of a human blood solution were used to investigate the cytotoxic activity of ME. The outcome shown that no turbidity development took place after adding 100 l of flavonide M. chamomile extract to all concentrations of human blood solution after 15, 30, and 60 min. This implies that the study's fungal extract did not have a cytotoxic effect (Figure 2).

Table 1. Molecular identification for keratinophilic fungi in our study (*the new keratinocyte fungal isolates in (DDBJ) for the first time ever)

Code	Molecular identification	Source	Accession number	strain
a	*Trichophyton rubrum*	skin	LC369522.1	TWCC 57922
b	*Trichophyton mentagrophytes*	skin	EF631618.1	BMU03104
c	*Microsporum canis*	skin	MT423731.1	19/600310
d	*Scopulariopsis brevicaulis*	nails	LC639848.1	9AFRODET*
e	*Scopulariopsis brevicaulis*	nails	LC639860.1	37AFRODET*
f	*Scupulariopsis brevicaulis*	nails	LR983931.1	CCF 6434
g	*Scopulariopsis cordiae*	nails	LC638843.1	2FRAFAF*
h	*Scopulariopsis stercoraria*	nails	LC638844.1	3AFFRAF*

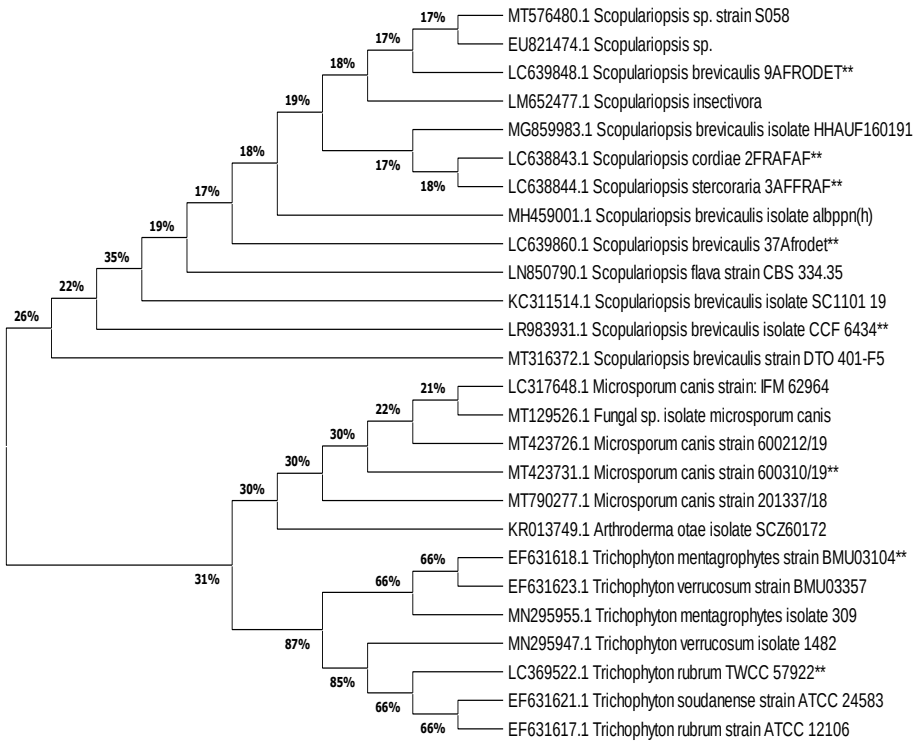

Figure 1. Phylogenetic tree of keratinophilic fungi (**our study strains).

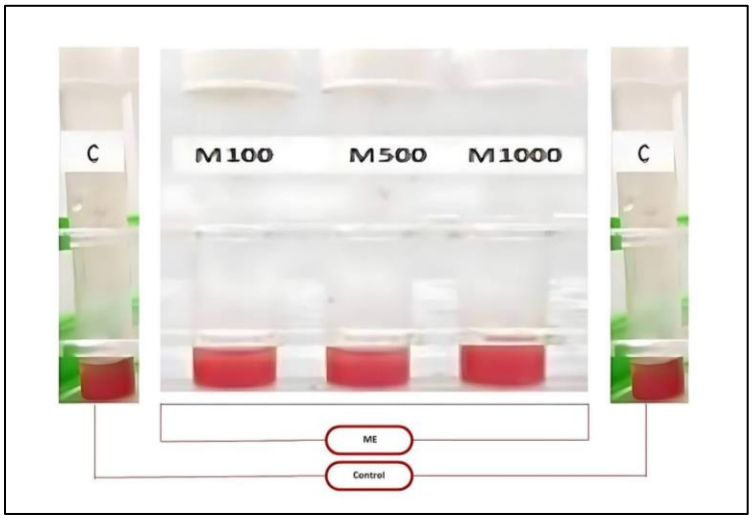

Figure 2. Toxicity test results for ME.

Antifungal Activity

Depending on the extract content and the source from which the sample was taken, the isolates had varying degrees of fungal growth inhibition. This implies that various fungi may be more or less susceptible to the extract, and that the origin of the sample may also affect how they react.

The methanolic extract showed a substantial inhibitory action against fungus in the efficacy test results. S. brevicaulis, one of the tested fungi, showed the most inhibition with a diameter of 26 mm at a dosage of 1000 g ml^{-1}, whilst M. canis showed the lowest inhibition with a diameter of 9 mm at a concentration of 100 g ml^{-1}. According to statistical analysis, there were highly significant differences between the concentrations utilized and the particular fungus species ($p< 0.05$), emphasizing the extract's varied effects. Particularly, S. brevicaulis demonstrated the extracts maximum receptivity (Table2, Figure 3, Figure 4). The M. chamomilla plant extract concentration of 1000 g ml^{-1} produced the maximum inhibition zone parameter. Additionally, statistical examination of the antifungal Nystatin indicated not important variations in the quantities utilized for most species.

GC-MS Profile of ME

In the current investigation, a sizable number of chemicals were found in the chamomile plant GC/MS analysis (Table 3) provides a summary of the significant elements and their percentages. There are at least 11 chemical compounds in M. chamomilla.

Table 2. Effectiveness of ME and the antifungal (Nystatin) on keratinophilic fungi isolates

Code	Control	Inhibition zone of ME (mm)				Inhibition zone of Nystatin(mm)			
		Concentrations g ml^{-1}				Concentrations g ml^{-1}			
		100	500	1000		100	500	1000	
a	0	13.00	15.00	19.00		.00	.00	.00	
b	0	10.00	12.90	15.00		.00	.00	.00	
c	0	6.00	12.00	16.00		.00	.00	.00	
d	0	18.00	22.00	26.00		.00	.00	.00	
e	0	18.00	22.00	26.00		.00	.00	.00	
f	0	17.00	20.00	26.00		.00	.00	.00	
g	0	10.00	13.90	19.00		18.00	21.00	24.00	
h	0	13.90	16.00	20.00		1200	15.00	20.00	

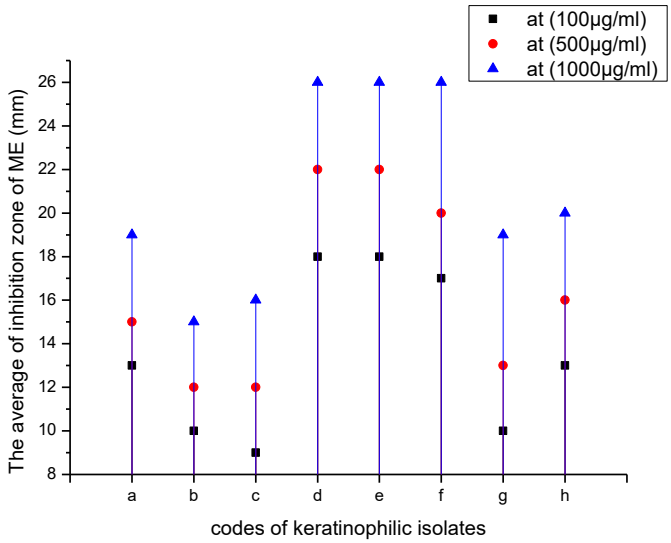

Figure 3. Effectiveness of ME on keratinophilic fungi isolates using some concentrations.

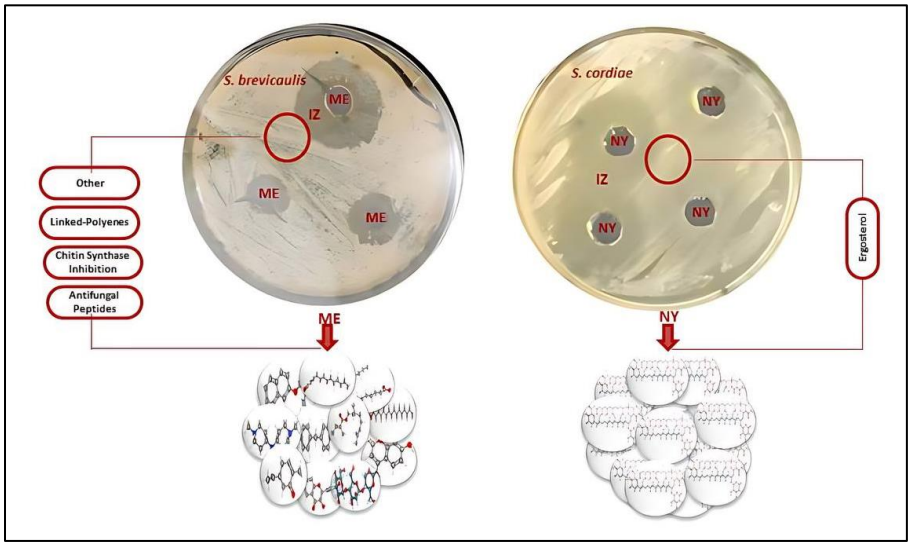

Figure 4. Inhibition Zone (IZ) and mechanism of action potential for ME and NY on keratinophilic fungi.

Table 3. Identifications, formulas, area, Retention Time (RT), Molecular Weight (MW) and chemical structures of GC-MS profile for ME

NO.	Name and Identification	Formula	Area (%)	RT (min)	M.W (g/mol)
1	2-Butoxynaphthalene	$C_{14}H_{16}O$	6.123	15.539	200.28
2	4-methylumbelliferone	$C_{10}H_8O_3$	5.123	16.049	176.17
3	Methyl hexadecanoate	$C_{17}H_{34}O_2$	5.83	17.724	270.5
4	oleic acid	$C_{18}H_{34}O_2$	8.232	18.505	282.5
5	4,4'-Methylenediphenol	$C_{13}H_{12}O_2$	5.83	19.411	200.23
6	Citronellyl tiglate	$C_{15}H_{26}O_2$	38.47	20.815	238.37
7	bis(1-methylpiperidin-4-yl)amine	$C_{12}H_{25}N_3$	1.3045	21.042	211.35
8	2-Tridecenal	$C_{13}H_{24}O$	1.1965	22.829	196.33
9	Fisetin	$C_{15}H_{10}O_6$	22.159	23.010	286.24
10	m-Cresol, 6-propyl-	$C_{10}H_{14}O$	2.411	23.665	150.22
11	Dextran	$C_{18}H_{32}O_{16}$	3.321	29.875	504.4

DISCUSSION

You used molecular techniques to identify the fungal isolates in our study. Due to its precision and dependability, this method is chosen above conventional morphological ones [22]. Given that it relies on visual observations of physical traits that could differ between species or be challenging to distinguish, morphological identification can be time-consuming and inaccurate [23].

You decided to use molecular techniques, which offer a straightforward and accurate way of identification, to get around these restrictions. We amplified and sequenced the ITS region of the fungal isolates in particular. Between the rRNA genes in the fungal genome is the conserved ITS region, which is frequently used to identify fungi[24]. By amplifying and sequencing the ITS region, we were able to obtain precise and consistent results for identifying the fungal species. The ITS region serves as a molecular marker that contains both highly conserved regions, which facilitate amplification, and variable regions, which allow for species-specific identification [25].

The simplicity and dependability of molecular identification are improved by the availability of ITS region sequences in generic databases. In order to accurately identify the fungal species, these sequences are used as a reference for comparing and matching the acquired sequences [26].

Our study used molecular techniques and focused on the ITS region to genetically identify fungal isolates. This method allowed us to precisely and reliably identify the species that were present during our investigation. The findings illustrated the importance of genetic diagnosis using the ITS region in comprehending fungal variety and prevalence by demonstrating the prevalence of Scopulariopsis brevicaulis among the isolated fungi.

A number of infections, including nail infections, onychomycosis, and invasive fungal diseases in immunocompromised people have been linked to Scopulariopsis species, including Scopulariopsis brevicaulis [27,28]. Therefore, worries regarding the possible influence on human health are raised by the high incidence of Scopulariopsis brevicaulis found in this study.

Additionally, it is important to note the presence of dermatophyte species like Trichophyton rubrum, Trichophyton mentagrophytes, and Microsporum canis

because these fungi are the main culprits behind common fungal infections in humans like dermatophytosis (ringworm), tinea infections, and others [29]. These infections, which can affect the skin, hair, and nails, may have an impact on public health, particularly in areas where these fungi are common [29].

In general, the development of suitable diagnostic and treatment approaches can benefit from understanding the prevalence and distribution of fungus through molecular identification [30]. Based on the susceptibility patterns of the detected species, it enables medical professionals to precisely identify the fungi that are the cause of fungal illnesses and select the most effective antifungal treatments [31].

The extract can be regarded as safe and possibly employed as a therapeutic substitute for antifungal drugs that might have negative host-related side effects. These results concur with research done by Osman et al in 2016 [32]. It's likely that the study in question supported the idea that the fungus extract utilized in the current study is safe and might be a beneficial therapeutic alternative.

The fact that the extract used in this study can be described as a therapeutic alternative to antifungals with adverse side effects on the host" indicates that the M. chamomile extract is regarded as safe and may one day be employed as a therapeutic option for treating fungal infections. The extract may have advantages over traditional antifungal drugs, which frequently have unfavorable host side effects.

To learn more about the effectiveness, safety, and possible therapeutic uses of the M. chamomile extract in treating fungal infections, additional research, such as in vitro and *in vivo* investigations, would also be necessary. Last but not least, the M. chamomile extract used in the study is safe and might be used as a therapeutic substitute for antifungals with negative side effects. However, more in-depth research is required to support this claim and provide a thorough understanding of its potential advantages and disadvantages.

The acquired results show that, within the permitted dose range, all tested fungal isolates responded differently to various doses of the flavonoid extract taken from M. chamomilla. Al-Dabbagh *et al.* [33] findings are consistent with the ME ability to effectively control the fungus.

Antibiotics should never be used without contacting a doctor, as doing so can result

in fungal colonization of the injured area. This may account for changes in the fungi's sensitivity and resistance patterns. Antibiotics can be poisonous to fungi, but they can also create resistance in them to lessen their effects [34].

Overall, these results highlight the methanolic extract's strong inhibitory action against the investigated fungus. Higher concentrations of the extract typically result in bigger inhibition zones. The concentration of the extract had a substantial impact on the inhibition. The varying susceptibility of the fungal species also emphasizes how crucial it is to take into account the precise target organisms when evaluating the effectiveness of antifungal medicines. According to the findings, S. stercoraria and S. cordiae are specifically sensitive to methanolic extract and Nystatin, respectively. These results advance our knowledge of the potential uses of organic plant extracts and antifungal medications to treat fungus infections. To understand the underlying mechanisms and improve their application in clinical or agricultural contexts, more investigation and study are required.

Various mold species, including *aspergillus, fusarium, alternaria, rhizoctonia*, and *chaetomium*, have been documented to be susceptible to the antifungal effects of natural extracts and essential oils from aromatic and medicinal plants [35,36]. There has previously been information about a natural peptide from M. chamomilla flowers with broad-spectrum antifungal activity against human pathogenic molds and yeasts [37]. The rise of antifungal drug resistance in fungal species is considered to be an increasing challenge for the public health.

Chemical compounds, related to essential oils and phenolic and flavonoid, prevent fungal growth through the inhibition of mycelia, conidial production, and sporulation of fungi [38]. Essential compounds from different plants have been reported to inhibit the fungal pathogens of F. moniliforme, F. oxysporum, Rhizoctonia solani, A. solani, and Aspergillus sp [39]. Flowers of M. chamomilla have a blue essential oils content, ranging from 0.2 % to 1.9 %, which has been used in various applications [39].

Extracts and essential oils of M. chamomilla exhibit a wide range of biological activities, such as antimicrobial, antioxidant and anti-inflammatory properties [40]. Plants and other naturally

occurring sources typically contain natural chemicals with biological activity [41].

The results of the current study showed that ME extract for M. chamomilla flowering contains a number of major bioactive compounds, which are believed to be responsible for its potent antifungal activity against all of the test fungal pathogens [42-45].

CONCLUSION

Our study emphasizes the value of molecular identification in comprehending the diversity and frequency of fungi. Our study's discovery of pathogenic fungus stresses the potential harm to human health and the demand for efficient diagnostic and therapeutic methods. The M. chamomilla extract demonstrated encouraging findings as a secure and effective substitute for conventional antifungal medications, but additional study is necessary to fully understand its therapeutic potential and mechanisms of action. In general, our research adds to the corpus of information on identifying fungi and creating antifungal tactics.

ACKNOWLEDGMENT

We would like to express our gratitude to everyone who assisted us in conducting our research.

REFERENCES

[1]. Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, Marks R, Naldi L, Weinstock MA, Wulf SK, Michaud C, J.L. Murray C, Naghavi M. An analysis of the prevalence and impact of skin conditions. *J Invest Dermatol*, 2014; 134(6): 1527-34.

[2]. White TC, Findley K, Dawson TL, Scheynius A, Boekhout T, Cuomo CA, Xu J, Saunders CW. Fungi on the skin: dermatophytes and Malassezia. *Cold Spring Harb Perspect Med*, 2014; 4(8): 19802.

[3]. Rouzaud C, Chosidow O, Brocard A, Fraitag S, Scemla A, Anglicheau D, Bouaziz J-D, Dupin N, Bougnoux M-E, Hay R, Lortholary O, Lanternier F, Group FMS. Severe dermatophytosis in solid organ transplant recipients: A French retrospective series and literature review. *Transpl Infect Dis*, 2018; 20(1): 12799.

[4]. de Hoog S, Monod M, Dawson T, Boekhout T, Mayser P, Gräser Y.

Skin Fungi from Colonization to Infection. *The Fungal Kingdom,* 2017, 855-71.

[5]. de Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D, Hendrickx M, Kupsch C, Stielow JB, Freeke J, Göker M, Rezaei-Matehkolaei A, Mirhendi H, Gräser Y. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. *Mycopathologia,* 2017; 182(1): 5-31.

[6]. Verma SB. Emergence of recalcitrant dermatophytosis in India. *Lancet Infect Dis,* 2018; 18(7): 718-19.

[7]. Bishnoi A, Vinay K. Dogra S. Emergence of recalcitrant dermatophytosis in India. *Lancet Infect Dis,* 2018; 18(3): 250-51.

[8]. Lockhart SR,Guarner J. Emerging and reemerging fungal infections. *Semin Diagn Pathol,* 2019; 36(3): 177-81.

[9]. Telles DR, Karki N, Marshall MW. Oral fungal infections: Diagnosis and Management. *Dent Clin N Am,* 2017; 61(2): 319-49.

[10]. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal drug resistance: molecular mechanisms in Candida albicans and beyond. *Chem Rev,* 2021; 121(6): 3390-3411.

[11]. Van Daele R, Spriet I, Wauters J, Maertens J, Mercier T, Van Hecke S, Brüggemann R. Antifungal drugs: What brings the future? *Med Mycol,* 2019; 57: 328-43.

[12]. Prasad R, Nair R, Banerjee A. Multidrug transporters of Candida species in clinical azole resistance. *Fungal Genet Biol,* 2019; 132(1): 103252.

[13]. Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. *Lancet Infect Dis,* 2017; 17(12): 383-92.

[14]. Tang S-S, Prodhan ZH, Biswas SK, Le C-F, Sekaran SD. Antimicrobial peptides from different plant sources: Isolation, characterisation, and purification. *Phytochem,* 2018; 154(2): 94-105.

[15]. Matić IZ, Juranić Z, Šavikin K, Zdunić G, Nađvinski N, Gođevac D. Chamomile and marigold tea: Chemical characterization and evaluation of anticancer activity. *Phytother Res,* 2013; 27(6): 852-58.

[16]. Aljazy NAS, Al-Mossawi AE-BHJ, Al-Rikabi AK. Study of antibacterial activity of some date seed extracts. *Bas J Agr Sci*, 2019; 32: 247-57.

[17]. Al-Mahmoud W. Novel variants of the DNA damage checkpoint protein Hus1 in fission yeast and human cells. *School of Biology Science, Bangor University United Kingdom. 2*014.

[18]. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR *Protocols,* 1990; 18(1): 315-22.

[19]. Srivastava JK and Gupta S. Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. *Mol Cell Pharmacol*, 2009; 1(3): 138.

[20]. Nair MG, Putnam AR, Mishra SK, Mulks MH, Taft WH, Keller JE, Miller JR, Zhu PP, Meinhart JD, Lynn DG. Faeriefungin: A new board-spectrum antibiotic from streptomyces griseus var. autotrophicus. *J Nat Prod,* 1989; 52(4): 797-809.

[21]. Perez C, Pauli M, Bazerque P. An antibiotic assay by the well agar method. *Acta Biol Med Exp,* 1990; 15(1): 113-15.

[22]. Kumar R, Gupta A, Srivastava S, Devi G, Singh VK, Goswami SK, Gurjar MS, Aggarwal R, Diagnosis and detection of seed-borne fungal phytopathogens. *Springer Singapore:* 2020, 107-142.

[23]. Arastehfar A, Wickes BL, Ilkit M, Pincus DH, Daneshnia F, Pan W, Fang W, Boekhout T. Identification of mycoses in developing countries. *J Fungus,* 2019; 5(4): 90.

[24]. Alrubayae IMN. Kadhim KF. Determination of oleaginous from non-oleaginous fungi using enzymatic and microscopic techniques. *IOP Conf Ser: Mat Sci Eng*, 2020; 928(6): 62011.

[25]. Tekpinar AD. Kalmer A. Utility of various molecular markers in fungal identification and phylogeny. Nova Hedwigia, 2019; 109(1-2): 187-224.

[26]. Stadler M, Lambert C, Wibberg D, Kalinowski J, Cox RJ, Kolařík M, Kuhnert E. Intragenomic polymorphisms in the ITS region of high-quality genomes of the

Hypoxylaceae (Xylariales, Ascomycota). *Mycol Prog,* 2020; 19(3): 235-45.

[27]. Iwen PC, Schutte SD, Florescu DF, Noel-Hurst RK, Sigler L. Invasive Scopulariopsis brevicaulis infection in an immunocompromised patient and review of prior cases caused by Scopulariopsis and Microascus species. *Med Mycol,* 2012; 50(6): 561-69.

[28]. Dellière S, Rivero-Menendez O, Gautier C, Garcia-Hermoso D, Alastruey-Izquierdo A, Alanio A. Emerging mould infections: Get prepared to meet unexpected fungi in your patient. *Med Mycol,* 2019; 58(2): 156-62.

[29]. Ebrahimi M, Zarrinfar H, Naseri A, Najafzadeh MJ, Fata A, Parian M, Khorsand I, Novak Babič M. Epidemiology of dermatophytosis in northeastern Iran; A subtropical region. *Curr Med Mycol,* 2019; 5(2): 16-21.

[30]. Millon L, Scherer E, Rocchi S, Bellanger A-P. Molecular Strategies to Diagnose Mucormycosis. *J Fungus,* 2019; 5(1): 24.

[31]. Reinel D. Non-dermatophyte fungi in onychomycosis—Epidemiology and consequences for clinical practice. *Mycoses,* 2021; 64(7): 694-700.

[32]. Osman MY, Taie HA, Helmy WA, Amer H. Screening for antioxidant, antifungal, and antitumor activities of aqueous extracts of chamomile (Matricaria chamomilla). *Egypt Pharm J,* 2016; 15(2): 55.

[33]. Al-Dabbagh B, Elhaty IA, Elhaw M, Murali C, Al Mansoori A, Awad B, Amin A. Antioxidant and anticancer activities of chamomile (Matricaria recutita L.). *BMC Res Notes,* 2019; 12(1): 3.

[34]. Denning DW. Echinocandin antifungal drugs. *Lancet,* 2003; 362(9390): 1142-51.

[35]. Behiry SI, Okla MK, Alamri SA, EL-Hefny M, Salem MZM, Alaraidh IA, Ali HM, Al-Ghtani SM, Monroy JC, Salem AZM. Antifungal and antibacterial activities of musa paradisiaca L. peel extract: HPLC analysis of phenolic and flavonoid contents. *Processes,* 2019; 7(4): 215.

[36]. Okla MK, Alamri SA, Salem MZM, Ali HM, Behiry SI, Nasser RA,

Alaraidh IA, Al-Ghtani SM, Soufan W. Yield, phytochemical constituents, and antibacterial activity of essential oils from the leaves/twigs, branches, branch wood, and branch bark of sour orange (Citrus aurantium L.). *Processes*, 2019; 7(6): 363.

[37]. Seyedjavadi SS, Khani S, Eslamifar A, Ajdary S, Goudarzi M, Halabian R, Akbari R, Zare-Zardini H, Imani Fooladi AA, Amani J, Razzaghi-Abyaneh M. The antifungal peptide MCh-AMP1 derived from matricaria chamomilla inhibits Candida albicans growth via inducing ROS generation and altering fungal cell membrane permeability. *Front Microbiol*, 2020; 10(1).

[38]. Salem MZM, Behiry SI, Hefny M. Inhibition of Fusarium culmorum, Penicillium chrysogenum and Rhizoctonia solani by n-hexane extracts of three plant species as a wood treated oil fungicide. *J Appl Microbiol*, 2019; 126(6): 1683-99.

[39]. Hamad Y, Abobakr Y, Salem MZM, Ali HM, Al-Sarar AS, Al-Zabib AA. Activity of plant extracts/essential oils against Three plant pathogenic fungi and mosquito larvae: GC/MS analysis of bioactive compounds. *BioRes*, 2019; 14(2): 23.

[40]. Mohamedsalih PM, Sabir DK. Biosynthesis of silver nanoparticles using the aqueous extract of chamomile flower and their antibacterial activity against Acinetobacter spp. *Health Biotechnology and Biopharma*, 2020. 3(4); 48-62.

[41]. Jafari-Sales A, Hossein-Nezhad P, Bolouri P. Identification of chemical composition of essential oil and evaluation of antimicrobial effects of ethanolic extract of Mentha pulegium on Staphylococcus aureus and Escherichia coli. *Health Biotechnology and Biopharma*, 2019. 3(1); 29-38.

[42]. Hwang IG, Kim HY, Woo KS, Lee SH, Lee J, Jeong HS. Isolation and Identification of the Antioxidant DDMP from Heated Pear (Pyrus pyrifolia Nakai). *Prev Nutr Food Sci*, 2013; 18(1): 76-9.

[43]. Tomić M, Popović V, Petrović S, Stepanović-Petrović R, Micov A, Pavlović-Drobac M, Couladis M.

Antihyperalgesic and antiedematous activities of bisabolol-oxides-rich matricaria oil in a rat model of inflammation. *Phytother Res*, 2014; 28(5): 759-66.

[44]. Ali A, Javaid A, Shoaib A. GC-MS analysis and antifungal activity of methanolic root extract of Chenopodium album against Sclerotium rolfsii. *Planta Daninha*, 2017; 35(1): 1-8.

[45]. Bhat MY, Talie MD, Wani AH, Lone BA. Chemical composition and antifungal activity of essential oil of Rhizopogon species against fungal rot of apple. *J Appl Biol Sci*, 2020; 14(3): 296-308.