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Metabolomic Profiling of Autotoxic and Autoprotective Compounds in Clinical *Candida albicans* Isolates via Gas Chromatography–Mass Spectrometry (GC–MS)

Sushree Swagatika Subhadarsini[1], Gopal Krishna Purohit[2]*, Lalli Smurthi Sahoo[3], Monali Priyadarsini Mishra[1], Pradipta Kumar Pati[4], Shreeram Behera[2]

[1]School of Allied and Healthcare Sciences, Centurion University of Technology and Management, Bhubaneswar, India

[*2]Heredity Biosciences, Plot No-818, Mayfair Lagoon Road, Jayadev Vihar, Bhubaneswar, Odisha, India

[3]Department of Microbiology, IMS and SUM Hospital Campus-2, Phulnakhara, Cuttack.

[4]Christ College, Kanika Rd, Near Chandi, Masik Patna Colony, Cuttack- 753008

***Corresponding Author:**

Dr. Gopal Krishna Purohit

Email ID: gopalpurohit2000@gmail.com

ABSTRACT

Candida albicans is a prominent opportunistic pathogen, whose persistence in clinical environments may be influenced by its metabolic secretions. This study used gas chromatography–mass spectrometry (GC-MS) to investigate the exometabolite profiles of clinical *Candida albicans* isolates from symptomatic female patients clinically diagnosed with vulvovaginal candidiasis (VVC) in hospitals across Odisha, India. Clinical symptoms included vaginal itching, discharge, burning sensation, and dysuria. After biochemical verification and culturing, the ethyl acetate extracts were subjected to gas chromatography-mass spectrometry (GC-MS) to identify volatile and semi-volatile compounds.

Metabolites were categorized as autotoxic compounds for self-regulation and autoprotective metabolites for stress adaptation and environmental resilience. The autotoxic metabolites included 2,4-di-tert-butylphenol (RT: 25.237 min), phthalate derivatives (RT: 33.104, 34.284 min), cyclotetrasiloxane (RT: 6.421 min), methylated alkanes, and dotriacontane (RT: 35.402, 36.557 min), which are known for their cytotoxic properties, suggesting their role in autolysis. The autoprotective profile included squalene (RT: 45.370 and RT: 45.444 min), methyl stearate, 13-docosenamide (Z), glycerol monostearate (TMS derivative), long-chain alkanes, and cyclic siloxanes, which are implicated in membrane stabilization and stress resistance.

The identification of both harmful and protective metabolites in Candida albicans reveals a complex self-regulatory mechanism that contributes to its pathogenicity and resistance to antifungal drugs. These findings suggest that the disruption of this metabolic balance could be a potential approach for antifungal treatment. Future studies should focus on quantifying these metabolites under stress conditions and evaluating their role in antifungal resistance.

Keywords: *Candida albicans; GC-MS; autotoxic metabolites; autoprotective metabolites; vulvovaginal candidiasis, metabolomics, antifungal resistance*

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1. INTRODUCTION

Candida albicans is a fungal pathogen that acts opportunistically, causing a range of infections, from mild mucosal candidiasis to severe systemic diseases, especially in individuals with weakened immune systems, such as those in intensive care units or HIV/AIDS [1-3]. Its ability to adapt is supported by a sophisticated regulatory system that includes morphological changes, biofilm development, and metabolic flexibility [4,5]. Notably, the self-regulation of metabolism through secreted endogenous substances deserves further investigation as it could uncover new targets for antifungal treatments.

Metabolomics enables a comprehensive snapshot of the small-molecule complement (metabolome) within and around microbial cells [6,7]. In fungal systems, gas chromatography–mass spectrometry (GC–MS) has been employed to quantify both intracellular and extracellular metabolites, providing insights into physiological and stress-response pathways [8]. Untargeted GC–MS studies of *Candida* species have revealed over 100 volatile and semi-volatile metabolites, including acids, alcohols, hydrocarbons, siloxanes, ketones, and phenolic compounds, which form a species-specific metabolomic signature [9]. However, the functional roles of these exometabolites, particularly in self-toxicity (autotoxicity) or self-protection, remain underexplored in clinical isolates.

Autotoxic metabolites inhibit the growth and viability of their producers and potentially function as quorum regulators, population control agents, or stress-induced self-disruptors- [10]. In contrast, autoprotective metabolites enhance stress resistance, membrane integrity, and oxidative defense, facilitating survival under hostile conditions such as antifungal exposure or host immune responses [11].

A prominent example is 2,4-di-tert-butylphenol (2,4-DTBP), a lipophilic phenol produced by bacteria, fungi, and plants with broad-spectrum toxicity, including cytotoxic, herbicidal, and antimicrobial effects [12]. Notably, 2,4-DTBP has demonstrated potent antifungal and antibiofilm activities against *Candida albicans*, inhibiting virulence traits such as hyphal differentiation and protease secretion [10, 11]. The dual nature of 2,4-DTBP, both self-produced and self-inhibitory, makes it emblematic of endocidal regulatory compounds [9, 12]. However, the natural occurrence and concentration dynamics of *Candida albicans* remain unclear.

Despite the potential significance of autotoxic/autoprotective molecules, most studies have focused on laboratory strains or dual-species biofilm models, often overlooking clinical isolates from diverse regions. For instance, untargeted GC–MS profiling of *Candida albicans–Klebsiella pneumoniae* biofilms revealed dynamic secretion of central carbon, amino acids, and secondary metabolites during biofilm maturation [13]; however, self-secreted autotoxic agents were not evaluated.

Our study analyzed extracellular ethyl acetate-extracted metabolites from *Candida albicans* isolates from different hospitals in Odisha, India. Using GC-MS, we identified volatile and semi-volatile compounds with potential autotoxic or autoprotective functions across retention time (5-50 min). Molecules such as phenols, phthalate esters, alkanes, fatty acid esters, squalene, and siloxanes are important for membrane structure modulation, oxidative stress mitigation, and self-inhibition. Clinical isolates of *Candida albicans* release self-toxic and self-protective metabolites, indicating a self-regulatory system for pathogenic resilience. This study identified antifungal targets by analyzing the bioactivity of compounds, including cytotoxicity, membrane protection, and quorum quenching. Increasing the number of self-toxic compounds or disrupting the protective metabolite pathways may provide new therapeutic strategies. We established a metabolic baseline for clinical isolates from Odisha to guide research on quantification under stress conditions and its correlation with biofilm formation, antifungal resistance, and virulence.

2. MATERIALS AND METHODS

2.1 Clinical Sample Collection: Vaginal Swabs

Clinical isolates of *Candida albicans* were obtained from vaginal swabs of vulvovaginal candidiasis patients at tertiary care hospitals in Odisha, India. The study targeted adult females showing symptoms of candidal vaginitis, including itching, discharge, and burning sensations. The exclusion criteria were antifungal treatment within two weeks before sampling, pregnancy, or immunosuppressive conditions other than diabetes mellitus. Trained staff collected the samples using cotton swabs in a sterile setting. The swabs were inserted into the posterior fornix and rotated for 10–15 seconds for adequate sampling. Each swab was placed in sterile transport medium and delivered to the microbiology lab within two hours.

Upon arrival, the swabs were inoculated onto Sabouraud Dextrose Agar (SDA) with chloramphenicol and incubated at 37°C for 24–48 h. Colonies with characteristics suggestive of *Candida albicans*, including germ tube formation and Candida differentiation on CHROMagar, were subjected to further biochemical and molecular confirmation.

2.2 Biochemical and Molecular Confirmation of Isolates

Colonies suspected to be *Candida albicans*, grown on SDA, were first assessed through Gram staining, which showed typical budding yeast cells accompanied by pseudohyphae. Germ tube tests were performed using human serum at 37°C for 2–3 h, with germ tubes serving as indicators of *Candida albicans*. To improve specificity, the isolates were cultured on *CHROMagar*™ Candida differential agar, where *Candida albicans* usually forms light to medium green colonies (Figure-1). Molecular confirmation was achieved by PCR amplification of the internal transcribed spacer (ITS) region of rDNA using ITS1 and ITS4 primers. The amplified products were analyzed by 1.5% agarose gel electrophoresis and the sequences were compared with NCBI GenBank references for definitive confirmation.

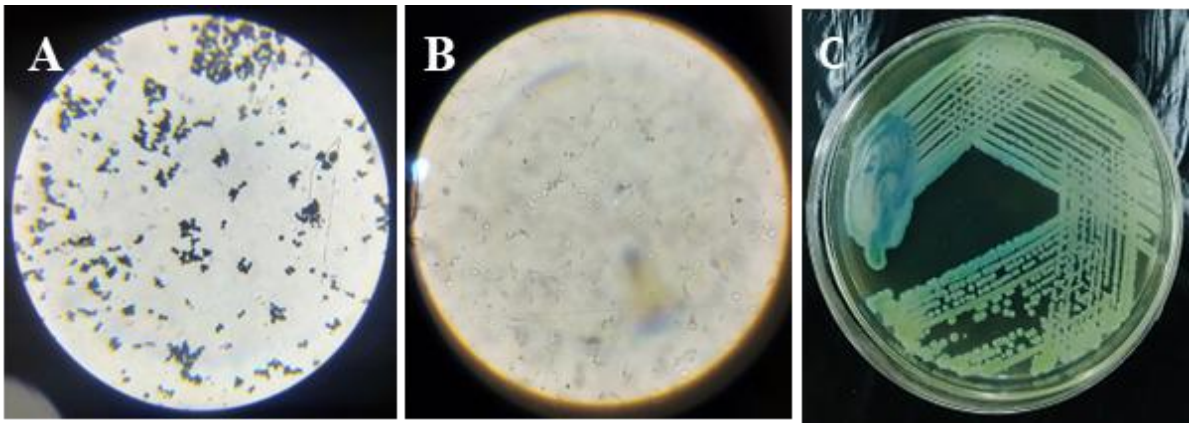


Figure 1. Phenotypic identification of *Candida albicans*: (A) Gram stain showing budding yeast with pseudohyphae; (B) germ tube formation in serum; (C) green colonies on CHROMagar™ Candida Differential Agar.

2.3 Culture and Metabolite Extraction

Candida albicans isolates were grown in Sabouraud Dextrose Broth (SDB) at 37°C for 48 h under static conditions to promote metabolite accumulation in the extracellular medium. After incubation, supernatants were collected by centrifugation at 5,000 rpm for 10 min and filtered through a 0.22 µm membrane to remove remaining cells. Extracellular metabolites were extracted through liquid-liquid partitioning using ethyl acetate in a 1:1 (v/v) ratio. The organic phase was evaporated to dryness using a rotary evaporator at 40 Â °C. The dried extracts were reconstituted in 1 mL of ethyl acetate and stored at −20°C until GC-MS analysis.

2.4 GC-MS Analysis

GC-MS profiling was performed using an Agilent Technologies GC system coupled with a mass-selective detector (7890 B GC with 5977A MSD). Separation was achieved on an HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness). The injection volume was 1 µL in splitless mode. The oven temperature was programmed to increase from 60°C (2 min hold) to 280°C at a rate of 10°C/min and held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1 ml/min.

Mass spectra were recorded in the 50–600 m/z range. Compound identification was performed by comparing the mass spectra to the NIST library database, and only compounds with a match probability ≥85% were considered for annotation.

2.5 Classification of Metabolites

The identified metabolites were categorized based on their structural types and documented biological activities, with particular attention paid to those recognized for their cytotoxic, membrane-disruptive, or antioxidant effects. To determine the functional role of each compound in the context of fungal physiology, literature reviews and publicly available chemical databases such as PubChem and ChemSpider were consulted.

2.6 Data Analysis and Interpretation

Following GC-MS analysis, the chromatograms and mass spectra were reviewed and cross-referenced using the NIST 2017 Mass Spectral Library. The retention times and mass spectral scores validated the identity of the compounds. Metabolites were tabulated using retention times, molecular weights, structures, and known bioactivities. Each metabolite was evaluated for autotoxic or autoprotective effects based on the literature on cytotoxicity, antimicrobial properties, antioxidant capacity, and biofilm modulation. Studies of similar compounds in fungal systems were prioritized, with predictions made from chemical classes for which functional data were unavailable.

2.7 Exometabolite Profiling of Clinical *Candida albicans* Isolates

This study focused on extracellular metabolites extracted using ethyl acetate and identified via GC-MS under specific conditions. Consequently, polar compounds and metabolites with low volatility or thermal instability may not be fully represented. This phase did not include quantitative analysis or functional validation such as cytotoxicity or membrane integrity assessments of the identified metabolites. These limitations will be addressed in subsequent research.

3. RESULTS

3.1 Isolation and Confirmation of *Candida albicans*

Of the 96 vaginal swab samples collected, 41 yielded isolates morphologically consistent with *Candida* spp. Among these, 30 isolates were confirmed as *Candida albicans* based on germ tube formation and characteristic green colony morphology on CHROMagar (Figure-1). PCR amplification of the ITS region confirmed the identity of 15 representative isolates with ≥98% sequence similarity with reference *Candida albicans* strains in the NCBI GenBank database.

3.2 GC-MS Profiling and Metabolite Identification

The ethyl acetate extracts of all 30 confirmed *Candida albicans* isolates were subjected to GC-MS analysis. Chromatograms revealed consistent metabolite profiles across the isolates, with variations in peak intensities. A total of 21 compounds were consistently detected and identified across multiple isolates based on retention time and mass spectral matching.

Metabolites were classified as autotoxic (cytotoxic, membrane-disruptive) or autoprotective (antioxidant, membrane-stabilizing, stress-adaptive), based on a literature review. Notably, 2,4-di-tert-butylphenol, phthalate esters, and dotriacontane are prominent autotoxic candidates, whereas squalene, glycerol monostearate, and cyclic siloxanes are categorized as autoprotective agents.

Table 1. Identified extracellular metabolites from clinical *Candida albicans* isolates by GC-MS and their putative biological roles

Compound Name	Retention Time (min)	Compound Class	Putative Role	Reference(s)
2,4-Di-tert-butylphenol	RT: 25.237	Phenol derivative	Autotoxic	[14]
Butyl hept-3-yl phthalate	RT: 33.104, 34.284	Phthalate ester	Autotoxic	[15]
Cyclotetrasiloxane, octamethyl-	RT: 6.421	Siloxane	Autoprotective	[16]
1-Nonadecene	RT: 32.110, 34.709	Alkene	Autotoxic	[17]
Methylated decane derivatives	RT: 10.187–10.458	Hydrocarbons	Autotoxic	[18]
Dotriacontane	RT: 35.402, 36.557	Long-chain alkane	Autotoxic	[19]
Hexadecanoic acid methyl ester	RT: 33.869	Fatty acid ester	Autotoxic	[20]
Methyl stearate	RT: 36.683	Fatty acid ester	Autoprotective	[21]
13-Docosenamide (Z-)	RT: 45.177	Fatty acid amide	Autoprotective	[22]
Glycerol monostearate (TMS derivative)	RT: 44.053	Monoacylglycerol	Autoprotective	[23]
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	RT: 42.659	Ester of fatty alcohol	Autoprotective	[24]
Tetracosane, Eicosane	RT: 31.304–34.789	Alkanes	Autoprotective	[25]
Cyclononasiloxane, octadecamethyl-	RT: 31.916, 40.617	Cyclic siloxane	Autoprotective	[26]
Tetracosamethyl-cyclododecasiloxane	RT: 42.173–47.479	Cyclic siloxane	Autoprotective	[27]
Squalene	RT: 45.370, 45.444	Triterpene	Autoprotective	[28]

3.3 Comparative Analysis of Metabolite Abundance

Visual inspection of the GC-MS chromatograms indicated considerable variation in peak intensity across isolates, suggesting differential metabolite secretion profiles among clinical *Candida albicans* strains. Although the qualitative profiles were conserved, certain metabolites appeared in higher abundance in subsets of isolates, potentially reflecting strain-specific metabolic strategies (Figure-2). The Total Ion Chromatogram (TIC) represents the summed intensities of all ions across the scan range, giving an overall profile where major peaks (32–37 min) indicate abundant compounds, such as fatty acid esters. In contrast, the Base Peak Chromatogram (BPC) recorded only the most intense ion per scan, producing sharper peaks that highlight dominant fragments, aiding the comparison of major lipid-like molecules (Supplementary data-1).

GC–MS Reported Compounds (with Molecular Weights) Extracted directly from the Candida albicans GC–MS Report. Values include derivatized compounds (e.g., TMS derivatives).

Compound Name	Formula	Molecular Weight (Reported)
Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	296
Undecane	C11H24	156
Decane	C10H22	142
Decane, 5-methyl-	C11H24	156
Hexane, 2,2,3,3-tetramethyl-	C10H22	142
Decane, 2,4-dimethyl-	C12H26	170
Nonane, 2,5-dimethyl-	C11H24	156
Undecane, 5-methyl-	C12H26	170
Nonane	C9H20	128
Decane, 4-methyl-	C11H24	156
Nonane, 2,6-dimethyl-	C11H24	156
Octane, 3,3-dimethyl-	C10H22	142
Heptane, 3,3,5-trimethyl-	C10H22	142
Nonane, 5-(2-methylpropyl)-	C13H28	184
Dodecane, 4,6-dimethyl-	C14H30	198
Undecane, 5,7-dimethyl-	C13H28	184
Octane, 6-ethyl-2-methyl-	C11H24	156
Dodecane, 2,6,10-trimethyl-	C15H32	212
Cyclohexasiloxane, dodecamethyl-	C12H36O6Si6	444
5-Amino-1-methyl-1H-pyrazole-4-carboxamide, 3TMS	C14H32N4OSi3	356
4-Amino-1,2-benzenediol, O,O',N-tris(trimethylsilyl)-	C15H31NO2Si3	341
Eicosane	C20H42	282
Hexadecane	C16H34	226
Heptadecane	C17H36	240
Heneicosane	C21H44	296
Heptadecane, 8-methyl-	C18H38	254
2,4-Di-tert-butylphenol	C14H22O	206
n-Pentadecanol	C15H32O	228
1-Nonadecene	C19H38	266
n-Heptadecanol-1	C17H36O	256
1-Hexadecanol	C16H34O	242
Decane, 1-iodo-	C10H21I	268
Triacontane	C30H62	422
2-Methylhexacosane	C27H56	380

11-Methyltricosane	C24H50	338
Tetrapentacontane	C54H110	758
Dotriacontane, 1-iodo-	C32H65I	576
Methyl tetradecanoate	C15H30O2	242
Tridecanoic acid, 12-methyl-, methyl ester	C15H30O2	242
Triacontanoic acid, methyl ester	C31H62O2	466
Tetracosanoic acid, methyl ester	C25H50O2	382
Cyclononasiloxane, octadecamethyl-	C18H54O9Si9	666
Cyclodecasiloxane, eicosamethyl-	C20H60O10Si10	740
Tetracosamethyl-cyclododecasiloxane	C24H72O12Si12	888
n-Nonadecanol-1	C19H40O	284
Behenic alcohol	C22H46O	326
9-Tricosene, (Z)-	C23H46	322
n-Tetracosanol-1	C24H50O	354
Dotriacontane	C32H66	450
Cyclo(L-prolyl-L-valine)	C10H16N2O2	196
Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione	C11H18N2O2	210
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O2	210
5-Azacytosine, N,N,O-trimethyl-	C6H10N4O	154
Tridecane, 4-methyl-	C14H30	198
Phthalic acid, hex-3-yl isobutyl ester	C18H26O4	306
Phthalic acid, hept-4-yl isobutyl ester	C19H28O4	320
Phthalic acid, isobutyl 2-pentyl ester	C17H24O4	292
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	278
Tetracosane	C24H50	338
Tetracontane	C40H82	562
Hexatriacontane	C36H74	506
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	276
Silane, trichlorooctadecyl-	C18H37Cl3Si	386
Silane, trichlorononyl-	C9H19Cl3Si	260
Hexadecanoic acid, methyl ester	C17H34O2	270
Pentadecanoic acid, methyl ester	C16H32O2	256
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	C18H28O3	292

Butylated Hydroxytoluene, TMS derivative	C18H32OSi	292
Pentatriacontane	C35H72	492
Tetratetracontane	C44H90	618
Hexacosane, 1-iodo-	C26H53I	492
Phthalic acid, butyl hept-3-yl ester	C19H28O4	320
Phthalic acid, butyl 6-methylhept-2-yl ester	C20H30O4	334
Phthalic acid, butyl hept-4-yl ester	C19H28O4	320
Dibutyl phthalate	C16H22O4	278
Phthalic acid, butyl 2-pentyl ester	C17H24O4	292
Eicosyl methyl ether	C21H44O	312
Cyclooctasiloxane, hexadecamethyl-	C16H48O8Si8	592
Tetrapentacontane, 1,54-dibromo-	C54H108Br2	914
2-Methyltetracosane	C25H52	352
Nonyl octacosyl ether	C37H76O	536
Hexacosyl nonyl ether	C35H72O	508
Nonyl tetracosyl ether	C33H68O	480
1-Decanol, 2-hexyl-	C16H34O	242
Heptadecane, 3-methyl-	C18H38	254
5,5-Diethylpentadecane	C19H40	268
Methyl stearate	C19H38O2	298
Heptadecanoic acid, 16-methyl-, methyl ester	C19H38O2	298
4-Nitrophenethylamine, N,N-bis-trimethylsilyl-	C14H26N2O2Si2	310
Octopamine, 4TMS derivative	C20H43NO2Si4	441
Silanamine, N-[1-[3-methoxy-4-[(trimethylsilyl)oxy]phenyl]-2-[(trimethylsilyl)oxy]ethyl]-	C21H45NO3Si4	471
Norepinephrine, (R)-, 5TMS derivative	C23H51NO3Si5	529
L-Isoserine, N,N,O-tri(trimethylsilyl)-, trimethylsilyl ester	C15H39NO3Si4	393
1-Heptacosanol	C27H56O	396
Methyl tetracosyl ether	C25H52O	368
Eicosyl isopropyl ether	C23H48O	340
6,6-Diethylhoctadecane	C22H46	310
6-Bromohexanoic acid, 5-ethyl-3-octyl ester	C16H31BrO2	334
6-Bromohexanoic acid, 4-hexadecyl ester	C22H43BrO2	418
Tridecane, 1,13-dibromo-	C13H26Br2	340
6-Bromohexanoic acid, 4-tridecyl ester	C19H37BrO2	376

N-Isopropyl-2-methyl-1-(2-methylbutyl)-4-(methylsulfonyl)-6-oxopiperazine-2-carboxami	C15H29N3O4S	347
Nonacosane	C29H60	408
1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	C16H26OSi	262
Acridin-9-yl-[1,2,4]triazol-4-yl-amine	C15H11N5	261
1,7-Bis(4-hydroxyphenyl)-5-methoxyheptan-3-one, 2TMS derivative	C26H40O4Si2	472
Hydrobenzoin, 2TMS derivative	C20H30O2Si2	358
Platyphyllonol, 3TMS	C28H46O4Si3	530
[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	C28H42O2	410
S-Indacene, 1,2,3,5,6,7-hexahydro-1,1,5,5-tetramethyl-4,8-bis(3-methylbutyl)-	C26H42	354
Pentafluorobenzyl alcohol, triisobutylsilyl ether	C19H29F5OSi	396
2,7-Dibutoxy-fluoren-9-one oxime	C21H25NO3	339
13-Methylheptacosane	C28H58	394
1-Hexacosanol	C26H54O	382
Bacteriochlorophyll-c-stearyl	C52H72MgN4O4	840
Octacosanol	C28H58O	410
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C14H16N2O2	244
Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alp	C33H37N5O5	583
3-Benzylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione, Ac derivative	C16H18N2O3	286
1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,19,19,19-docosamethyldecasiloxane	C22H66O9Si10	754
1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17-icosamethylnonasiloxane	C20H60O8Si9	680
1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-octadecamethyloctasiloxane	C18H54O7Si8	606
Heptasiloxane, hexadecamethyl-	C16H48O6Si7	532
2-Methylheptacosane	C28H58	394
1,3,5-Trisilacyclohexane	C3H12Si3	132
3-(Hexadecyloxy)propan-1-ol, TMS	C22H48O2Si	372
1,3-Dipalmitin, TMS derivative	C38H76O5Si	640
octadecanoic acid, 3-oxo-, ethyl ester	C20H38O3	326
3-Hydroxypropyl palmitate, TMS derivative	C22H46O3Si	386
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330

Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	358
Glycerol 1-palmitate	C19H38O4	330
9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluo	C30H42Cl2N4O3	576
Bis(2-ethylhexyl) phthalate	C24H38O4	390
Diisooctyl phthalate	C24H38O4	390
6,6'-Methylenebis(2,4-di-tert-butylphenol)	C29H44O2	424
Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	C39H80O2	580
1,3-Propanediol, ethyl octadecyl ether	C23H48O2	356
1,3-Propanediol, docosyl ethyl ether	C27H56O2	412
Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy]methyl]ethyl ester	C55H106O6	862
Glycidyl palmitate	C19H36O3	312
Glycerol 2-acetate 1,3-dipalmitate	C37H70O6	610
Hexadecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	C23H42O6	414
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	C35H68O5	568
Octadecane, 2,6,10,14-tetramethyl-	C22H46	310
Nonadecane, 2,6,10,14-tetramethyl-	C23H48	324
Hexacontane	C60H122	842
Glycerol monostearate, 2TMS derivative	C27H58O4Si2	502
3,7,11,15,18-Pentaoxa-2,19-disilaeicosane, 2,2,19,19-tetramethyl-	C17H40O5Si2	380
3,7,11,14,18-Pentaoxa-2,19-disilaeicosane, 2,2,19,19-tetramethyl-	C17H40O5Si2	380
13-Docosenamide, (Z)-	C22H43NO	337
9-Octadecenamide, (Z)-	C18H35NO	281
Tetratriacontane	C34H70	478
Squalene	C30H50	410
6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-	C30H51BrO	506
2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol	C30H54O2	446
1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C30H50O	426
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	C30H52O	428
.psi.,.psi.-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-	C40H66	546

Supraene	C30H50	410
5-Butyl-5-ethylheptadecane	C23H48	324
1-Propanol, 2,3-bis[(3,7,11,15-tetramethylhexadecyl)oxy]-	C43H88O3	652
1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane	C32H64	448
5,5-Diethylheptadecane	C21H44	296
6-Tetradecanesulfonic acid, butyl ester	C18H38O3S	334
Triacontane, 1,30-dibromo-	C30H60Br2	578
Pentacosane, 1-bromo-	C25H51Br	430
Triacontane, 1-bromo-	C30H61Br	500

In patients with recurrent vulvovaginal candidiasis (RVVC), the isolates showed higher levels of autotoxic substances, such as 2,4-di-tert-butylphenol and phthalate esters. Isolates from patients with diabetes contained dotriacontane and methylated alkanes, suggesting changes in lipid metabolism due to host pressure. Isolates from patients with prior antifungal exposure showed increased levels of autoprotective substances such as squalene, siloxanes, and fatty acid esters, indicating adaptive increases in membrane-protective metabolites.

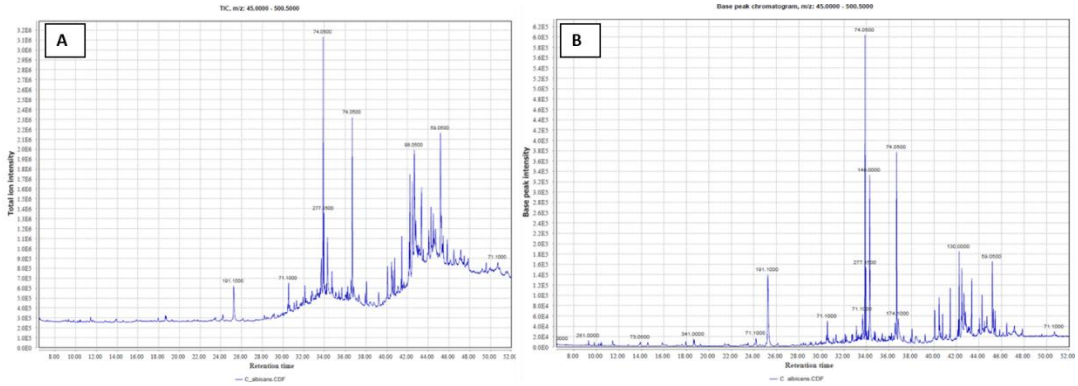


Figure 2. Representative GC-MS chromatogram of the ethyl acetate extract from a clinical *Candida albicans* isolate.

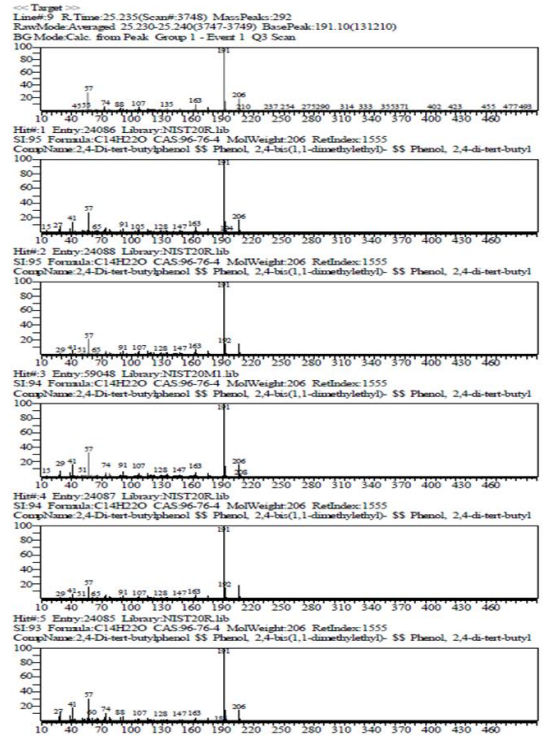
The major peaks were labelled with retention times and compound identities corresponding to the metabolites listed in Table 1. Peaks representing both autotoxic (e.g., 2,4-di-tert-butylphenol and phthalates) and autoprotective (e.g., squalene and siloxanes) compounds were marked for comparison.

3.4 Autotoxic and Autoprotective Profile Delineation

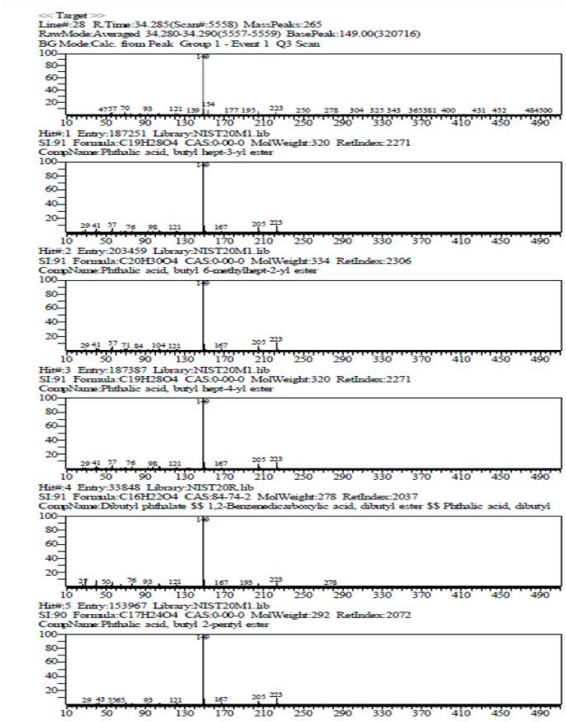
Based on relative peak areas and literature-validated roles, each isolate was assigned a semi-quantitative ratio of autotoxic to autoprotective metabolites by calculating the sum of the peak areas for the identified autotoxic vs. autoprotective compounds. Ratios above 1 indicated autotoxic predominance, whereas ratios below 1 indicated autoprotective bias.

Approximately 40% (12/30) of the isolates exhibited an autoprotective-dominant profile, with high levels of squalene, siloxanes, and fatty acid derivatives. The remaining isolates displayed either a balanced or autotoxic-biased profile with enriched 2,4-DTBP, phthalates, or long-chain alkanes.

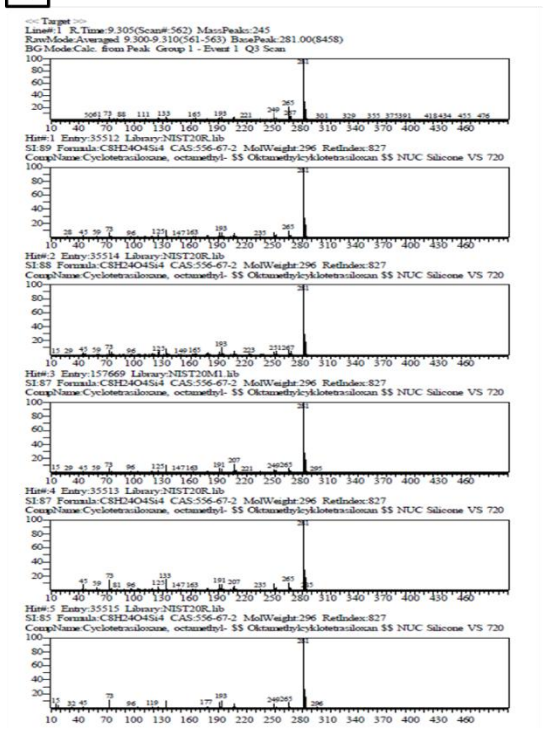
A **2,4-Di-tert-butylphenol, Peak(s): 25.237 (Peak #9), Formula: C14H22O, Molecular Weight: 206.33 g/mol**



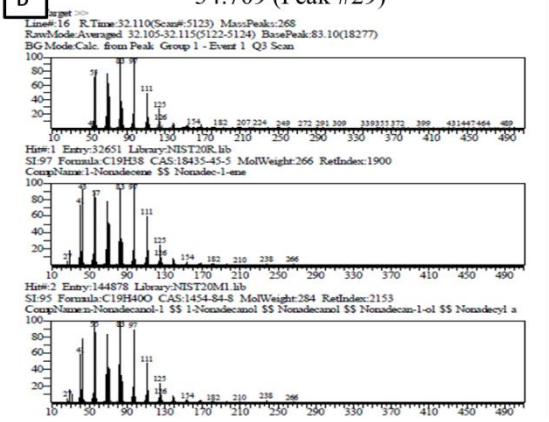
B **Butyl hept-3-yl phthalate, Peak(s): 33.104 (Peak #19), 34.284 (Peak #28)**



C Cyclotetrasiloxane, octamethyl-, Peak(s): 9.306 (Peak #1)

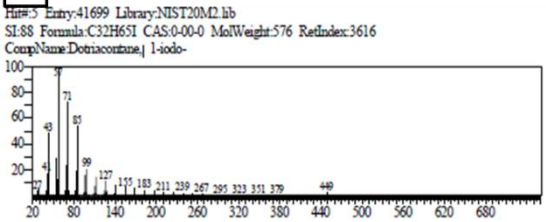


D 1-Nonadecene, Peak(s): 32.110 (Peak #16), 34.709 (Peak #29)



Dotriacontane, Peak(s): 35.402 (Peak #31), 36.557 (Peak #36), 40.071 (Peak #43), 41.444 (Peak #49), 42.483 (Peak #55), 44.267 (Peak #63)

E



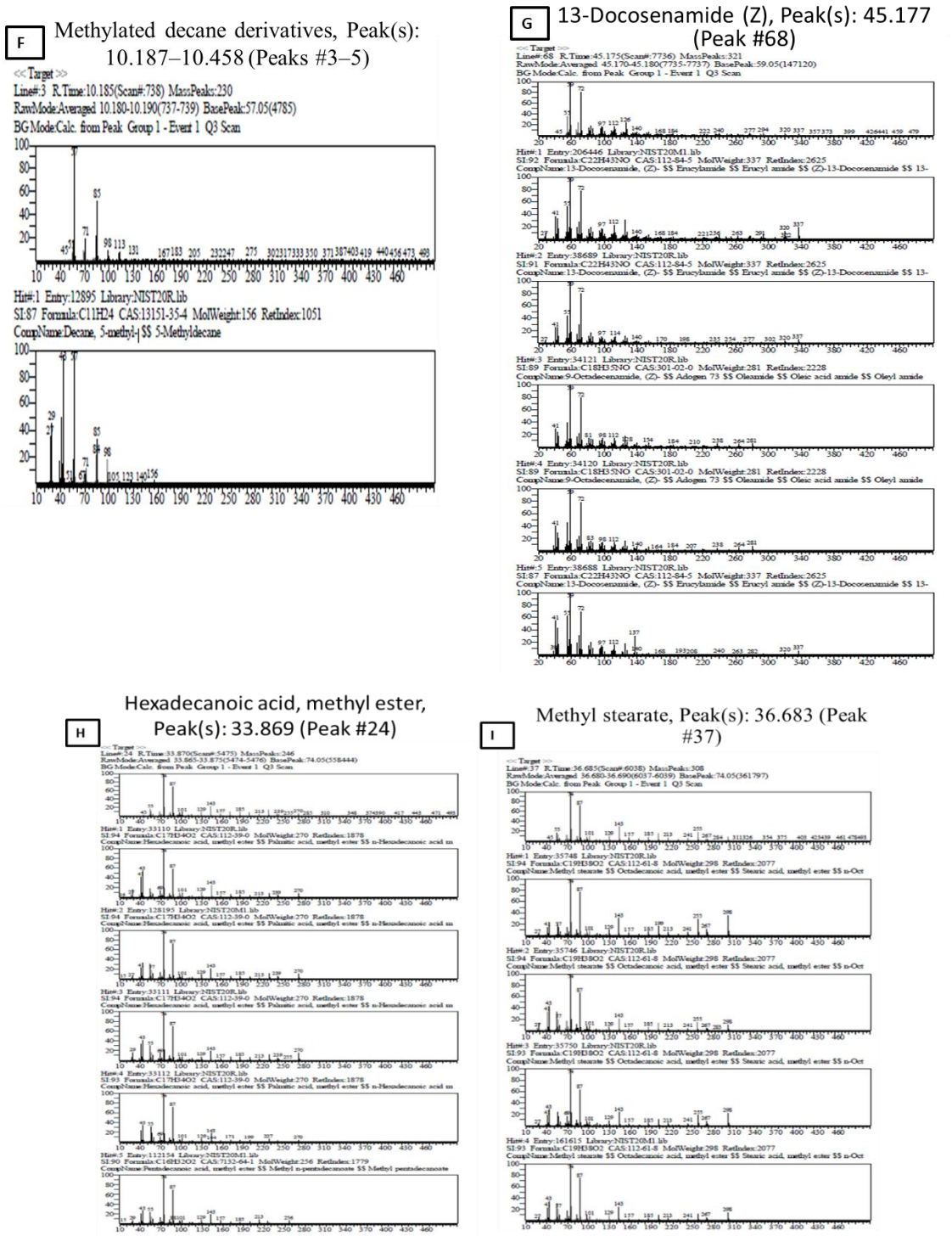


Figure 3: GC-MS profile of Candida albicans metabolites showing Total Ion Chromatogram (TIC), Base Peak Chromatogram (BPC), and mass spectra of major compounds. The peaks detected between 25-47 min correspond to phenolic derivatives, phthalates, hydrocarbons, alkanes, fatty acid esters, fatty acid amides, monoacylglycerols, cyclic siloxanes, and triterpenes. The identified metabolites (e.g., 2,4-di-tert-butylphenol, methyl stearate, and squalene) were classified as autotoxic or autoprotective based on their biological functions.

3.5 Cluster Analysis and Functional Grouping

Through classified clustering of the metabolite profiles of Candida albicans isolates, researchers have identified three distinct groups with specific exometabolomic signatures. Cluster A showed elevated levels of autoprotective metabolites, such as squalene and siloxanes, associated with prior antifungal exposure, suggesting an adaptation to drug pressure. Cluster B contained isolates with high autotoxic metabolites, notably 2,4-di-tert-butylphenol (2,4-DTBP) and phthalates,

predominantly in recurrent vulvovaginal candidiasis (RVVC) cases, indicating metabolic dysregulation. Cluster C was heterogeneous, with moderate levels of both autoprotective and autotoxic compounds, similar to the initial infection profiles (Table-2). These findings indicate that Candida albicans modifies its exometabolome in response to host signals and treatment history to enhance its persistence and resistance.

Table 2: Hierarchical Clustering of *Candida albicans* Isolates Based on Metabolite Profiles

Cluster	Dominant Metabolites	Metabolic Signature	Clinical Association	Interpretation
A	Squalene, Siloxanes	High autoprotective content	Associated with prior antifungal exposure	Indicative of adaptive response to pharmacologic stress
B	2,4-DTBP, Phthalates	High autotoxic load	Predominant in recurrent *vulvovaginal candidiasis* (RVVC) cases	Reflects metabolic stress or dysregulation under chronic infection
C	Moderate levels of both A & B metabolites	Mixed profile	Common in first-time infections	Suggests baseline metabolic state before treatment pressure

4. DISCUSSION

This study provides a comprehensive GC-MS-based metabolomic characterization of extracellular compounds secreted by clinical *Candida albicans* isolates obtained from patients with vulvovaginal candidiasis in Odisha, India. Our findings demonstrated the coexistence of both autotoxic and autoprotective metabolites in the secretome, highlighting a potentially complex self-regulatory network that supports *Candida albicans* survival, pathogenicity, and adaptation under varying host and environmental pressures.

A significant observation was the recurrent detection of 2,4-di-tert-butylphenol (2,4-DTBP) in most isolates. This phenolic compound is known for its antimicrobial, antibiofilm, and cytotoxic effects, including the inhibition of fungal hyphal formation, protease secretion, and modulation of oxidative stress [29,30]. In the context of *Candida albicans*, the endogenous secretion of such an autotoxic compound raises compelling questions regarding its physiological role. It is plausible that 2,4-DTBP participates in quorum sensing or density-dependent regulation, serving as a negative feedback molecule to control population expansion or induce autolysis under stress conditions [31]. A similar endocidal behavior has been described in bacterial systems, where toxic compounds are produced by the organisms themselves for regulatory functions [32].

The presence of phthalate esters such as butyl hept-3-yl phthalate further supports the possibility of autotoxic regulation. These compounds are associated with membrane disruptive properties and oxidative damage [33]. Their consistent presence suggests that *Candida albicans* may utilize such esters not only as metabolic by-products, but also as signaling agents or defense tools to modulate inter- or intra-species competition.

Conversely, the detection of metabolites such as squalene, methyl stearate, glycerol monostearate, and cyclic siloxanes suggests a robust autoprotective profile in several isolates. Squalene, a key intermediate in sterol biosynthesis, has been well-documented for its antioxidant and membrane-stabilizing properties, particularly under oxidative and antifungal stress [34]. The elevated secretion in isolates from patients with prior antifungal exposure supports the hypothesis of a protective adaptive response. Similarly, the cyclic siloxanes and fatty acid esters identified in this study have been linked to increased membrane fluidity and resistance to lipid peroxidation, further contributing to fungal resilience [35].

The dual presence of autotoxic and autoprotective metabolites implies dynamic metabolic equilibrium within the fungal microenvironment. Such a system could enable *Candida albicans* to fine-tune its physiological state based on host immune responses, antifungal exposure, and nutrient availability. In hostile environments, upregulation of autoprotective metabolites may confer survival advantages, whereas accumulation of autotoxic compounds may signal metabolic overburden, initiate programmed cell death, or regulate the community structure in biofilms.

Interestingly, isolates from patients with recurrent vulvovaginal candidiasis (RVVC) and diabetes exhibited a more autotoxic-biased profile, including higher levels of long-chain alkanes such as dotriacontane and 1-nonadecene. These hydrocarbons are associated with cytotoxicity and membrane perturbation [36], and their overproduction could either reflect dysregulated metabolism or play a functional role in population thinning or immune evasion. In contrast, isolates from patients with a history of antifungal treatment displayed an autoprotective-dominant profile, suggesting metabolic conditioning or selection for resilient strains.

Our clustering analysis further supports the idea of strain-specific metabolic adaptations. The formation of distinct

metabolomic clusters corresponding to treatment history and clinical status indicates that extracellular metabolite profiles could serve as biomarkers for virulence potential, treatment response, or infection chronicity. Such profiling could eventually aid in the design of precise antifungal therapies and diagnostics.

Although GC-MS enabled the detection of volatile and semi-volatile compounds, polar and non-volatile metabolites may have been under-represented. Moreover, we did not quantify the absolute concentrations of the metabolites or validate their biological activities using functional assays [37]. Future studies should focus on targeted metabolomics combined with bioassays to confirm the cytotoxicity, antioxidant activity, and immunomodulatory effects of these compounds.

Additionally, transcriptomic or proteomic integration could help elucidate the biosynthetic pathways responsible for the observed metabolite profiles and determine whether these pathways are regulated in response to host-derived signals or antifungal stress[37]. Exploring the interplay between metabolic output, biofilm formation, virulence gene expression, and drug resistance will provide deeper insights into the functional consequences of the secreted metabolome.

5. CONCLUSION

This study investigated the extracellular metabolomic profile of clinical isolates of Candida albicans, uncovering autotoxic and autoprotective substances in a self-regulatory mechanism. GC-MS analysis of the ethyl acetate extracts identified volatile and semi-volatile metabolites, including cytotoxic substances such as 2,4-di-tert-butylphenol, phthalates, and long-chain alkanes, as well as metabolites aiding stress resilience and membrane protection, such as squalene, siloxanes, and glycerol monostearate. Differences in metabolite profiles among isolates linked to patient clinical history suggest that Candida albicans modifies its secretome in response to environmental challenges. This adaptability may influence the persistence of the pathogen, immune evasion, and antifungal resistance. Our results highlight the importance of fungal metabolomics in understanding pathogenesis and treatment responses. The dual function of secreted metabolites as inhibitors and protectors presents opportunities for antifungal strategies such as disrupting protective pathways or enhancing self-toxic compounds. Further research is required to assess the biological activities of these metabolites and their interactions with gene expression and biofilm formation. Combining metabolomic data with genomic and proteomic analyses will deepen our understanding of fungal resilience mechanisms and aid in antifungal therapy development.

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Conflict of Interest

The authors declare no conflicts of interest.

Supplementary Data

The supplementary material features a table of compounds identified from extracellular extracts of *Candida albicans* through GC–MS analysis. This dataset includes retention times, molecular formulas, compound names, and molecular weights with identification based on matches. Several compounds, such as TMS derivatives, are present in derivatized forms, which as TMS derivatives, typical in standard GC–MS sample preparation methods.

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