

**ANTI CANDIDAL ACTIVITY OF GREEN TEA (*CAMELLIA SINENSIS*) EXTRACT
AGAINST MDR CANDIDA SP. ISOLATED FROM SOLID WASTE OF LOCAL MARKET****Smaranya Singha Roy¹, Aniruddha Mukherjee², Dr. Anirban Mukherjee^{2*}**¹Department of Pharmaceutics, Guru Nanak Institute of Pharmaceutical Science and Technology, 157/f Nilgunj Road, Sodepur, Panihati, West Bengal – 700114.²AM Educare Biotech Solutions Pvt Ltd, P 12 CIT Road, Kolkata – 700054.***Corresponding Author: Dr. Anirban Mukherjee**

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ABSTRACT

The increase of the irrational use of antifungal drug has been a major cause of the development of multidrug-resistant (MDR) *Candida* species, which represents a threat to the limited activity of conventional antifungals. The research article examines the possibility of using green tea extract as a natural phytochemical counter-measure of MDR *Candida* in planktonic and sessile form. In the present research, the selective agar was used to isolate *Candida* sp from solid waste disposal of local market and analyze its MDR through resistance profiling. The crude extract of *Camellia sinensis* (used as green tea) was applied in this experiment and are characterized using Thin Layer Chromatography (TLC). To check the efficiency, different drug screening assays were conducted, such as cup plate assay, MTT assay, MIC assay and the anti-biofilm assay. We have found that green tea bioactive compounds and more so catechins have an important role in disrupting the biofilm structure of fungi. Overall, this study recommends the application of the green tea extract as an effective, natural, and readily available intervention on the management of MDR *Candida* infection. We provide a new and efficient alternative, which can be used in clinical practice as a complementary or an alternative of the current antifungal treatments using the inherent antifungal properties of green tea.

KEYWORDS: Green tea extract; MDR *Candida* biofilms; Phytochemicals; Anti-biofilm; Antifungal Resistance.**1. INTRODUCTION**

Candida spp. intravascular infection is among the severe fungal opportunistic infections that is causing high mortality and morbidity of critically ill and immunocompromised patients particularly in the intensive care units. They are most frequently occurring as bloodstream infections that arise in approximately one-third of the cases and end with the fatality rate of 40-50% (Chaudhary et al., 2015; Clancy & Nguyen, 2018). The increasing occurrence of non-albicans species, which is resistant to different drugs, including cefepime and ceftriaxone, such as *C. glabrata*, *C. tropicalis*, and *C. aureus*, that result in nosocomial candida makes this problem more complicated (Fanning & Mitchell, 2012a). Consequently, the threat led to its designation as a critical-priority pathogen by the World Health.

Organization (WHO) in 2022 due to its overall prevalence and capability to cause epidemics, whereas other species, including *C. albicans*, are of high priority, and the necessity to come up with novel antifungal and anti-biofilm strategies is dire (Katsipoulaki et al., 2024). The weight is especially heavy in India. Approximately, 188,035 cases of ICU-associated candidemia are estimated annually, approximately 37,607 in ICU (6.5 per 1,000 ICU admissions) (Ray et al., 2022). Of interest, non-albicans species account for approximately 71% of invasive candidiasis infections in Indian hospitals, and biofilm-positive isolates account for a significant proportion of persistent infections (Samaddar et al., 2022). Studies from New Delhi investigations identified that 77.1% of candidemia isolates were biofilm-producing, 100% of *C. auris* and very high proportions of all other non-albicans species (Sheena & Singh, 2023).

The formation of *Candida* biofilm is a well-coordinated, multi-stage development process which is centrally involved in *Candida* virulence and *Candida albicans* and non-*albicans Candida* (NAC) species persistence in *Candida* infections (i.e. *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and recently emerged multidrug-resistant *Candida aureus*) (Fanning & Mitchell, 2012a; Gulati & Nobile, 2016; Kean et al., 2018). This is induced by attaching itself to the biotic and abiotic surfaces through an adhesin and it is largely regulated by the agglutinin-like sequence (ALS) protein family and hyphae wall protein 1 (Hwp1) that facilitates the colonisation of the stable surfaces and intercellular adhesion (Nobile et al., 2008). The filamentous species, including hygae and pseudohygae, become morphogenetically differentiated and proliferate as a result of adhesion to form a 3D biofilm structure (Fan et al., 2022a; Fanning & Mitchell, 2012b; Taff et al., 2013a). The process of maturation is linked to laying down of a complex extracellular matrix (ECM) of β -1,3-glucans, mannan, proteins, lipid and extracellular DNA that provide the environment with structural integrity and creates gradients of nutrients, oxygen and pH that allow the environment to support metabolic diversification and resistance to stress (Mitchell et al., 2016; Roy & Gow, 2023). The biofilm-linked antifungal resistance occurs due to physical, biochemical, and genetic mechanisms that are integrated together, reducing drug effectiveness by several orders of magnitude compared to planktonic cells (Taff et al., 2013b). The ECM seems to be a diffusion barrier and drug-sequestering scaffolds, especially by β -glucan-mediated binding of azoles and polyenes, which reduce the amount of drugs that are accumulated in the cell (Fan et al., 2022b; Sandai et al., 2016). At the same time, biofilm cells have a high expression of efflux transporters of ATP-binding cassette (ABC) and major facilitator superfamily (MFS), such as *CDR1*, *CDR2*, and *MDR1* which also actively release structurally different antifungal agents (Prasad et al., 2019). In biofilms, metabolic reprogramming is also important to tolerance because small groups of cells undergo slow-growing or persister responses that transiently show non-heritable resistance to fungicidal stress (LaFleur et al., 2006).

Quorum-sensing molecules including farnesol and tyrosol as well as hierarchical transcriptional regulatory networks of major regulators such as Bcr1, Efg1, Tec1, and Ndt80, coordinate these phenotypes collectively controlling adhesion, matrix biosynthesis, morphogenesis, and dispersal (Nobile et al., 2012; Rodrigues & Černáková, 2020). Notably, the species-specific variations in biofilm composition and regulatory structure as well as the efflux-dominant resistance of *C. glabrata* and the thick, environmentally adapted biofilms of *C. auris*, are highly troublesome to clinical management and highlight the need to develop multi-target, adjunctive anti-biofilm activity that is not confined to the paradigm of traditional antifungal agents (Kean et al., 2018; Ramage et al., 2014).

The formation of biofilms by the *Candida* spp. is a significant cause of invasive candidiasis in hospitals, especially when biofilms are formed on the indwelling devices such as central venous and urinary catheters, pacemakers, and prosthetic valves (Ajetunmobi et al., 2023; Desai et al., 2014a; Nobile & Johnson, 2015). These biofilms are persistent reservoirs that keep on seeding the blood and removal of the gadget is usually necessary to cure them. In India, a majority of the isolates of *Candida* are biofilm producers (more than 70%), with non-*albicans* species like *Candida tropicalis* and *Candida Auris* having very high prevalence (Chandra & Mukherjee, 2015; Desai et al., 2014b). Interestingly, the WHO Critical Priority pathogen, the *C. aureus*, develops compact, drug-resistant biofilms that cannot be eliminated even by using echinocandins.

Phytochemicals represent a heterogeneous group of plant-based secondary metabolites that have become promising antifungal and anti-biofilm agents due to their ability to interact with more than one cellular pathway at the same time, thus hindering immunisation against them (Zhou et al., 2023). Unlike classical antifungals, which tend to act on a single molecular target, a large number of phytochemicals, especially polyphenols, flavonoids, and terpenoids, are pleiotropic in action, disrupting membrane integrity, redox balance, and regulatory signalling networks in the species of the genus *Candida* (Cowan, 1999; Ramage et al., 2014). These compounds induce physicochemical interactions between themselves and the fungal cell membranes, making them more permeable and thus disrupting membrane-associated processes; in some cases, they disrupt the ergosterol synthesis, which increases susceptibility to azoles and polyenes (Pinto et al., 2006). At the biofilm scale, phytochemicals prevent the initial stages of adhesion and extracellular matrix (ECM) formation by interfering with the expression of surface adhesin and morphogenetic regulators, resulting in the disrupted biofilm establishment and decreased structural stability. In addition, disruption of quorum-sensing molecules like farnesol and tyrosol changes hyphal development and biofilm maturation, thus facilitating attenuated virulence and high dispersal (Shankar Raut & Mohan Karuppayil, 2016). One such clinically relevant property of a variety of plant-derived compounds is their ability to inhibit multidrug resistance (MDR) efflux systems, such as ATP-binding cassette and major facilitator superfamily transporters, increasing intracellular levels of antifungal agents and partially restoring drug susceptibility in resistant isolates of *Candida aureus* (Prasad et al., 2019; Stermitz et al., 2000). Moreover, some polyphenols have pro-oxidant activity in vivo that causes the production of intracellular reactive oxygen species (ROS) which overwhelms fungal defences against antioxidants, and activates apoptosis-like cell death mechanisms. All these multimodal activities create a mechanistic basis for the use of phytochemicals as adjunctive, biofilm-targeted therapies and offer a strong argument for considering catechin-rich extracts of *Camellia sinensis* as

complementary interventions with multidrug-resistant biofilms of *Candida*.

Epigallocatechin gallate (EGCG) is the major catechin in *Camellia sinensis*, which is a rational anti-biofilm therapy against multidrug-resistant strains of *Candida* because it interferes with essential metabolic and structural mechanisms (Evensen & Braun, 2009). EGCG acts as an antifolate by blocking the dihydrofolate reductase (DHFR; Ki 0.7mM) as well as depleting folate-dependent pools of S-adenosyl-methionine indirectly by blocking sterol C-24 methyltransferase (Navarro-Martínez et al., 2006). Simultaneously, EGCG specifically interacts with the ergosterol in the fungal membrane and inhibits the processes of proteasomes, which are chymotrypsin-like and peptidyl-glutamyl-peptide hydrolysing, causing structural damage and the inability to maintain bacterial biomass in the biofilm. By such concerted efforts, EGCG prevents biofilm formation as well as substantially reduces established biofilms in a dose-dependent fashion with reported decreases of up to about 80% in all *Candida* species, which is why it is considered a promising anti-biofilm agent against drug-resistant isolates (Guida et al., 2013). In spite of the great progress in studying *Candida* biofilm biology and the emergent role of plant-derived anti-biofilm compounds as adjunctive antifungal agents, the systematic assessment and mechanistic characterisation of the translational placement of plant-derived anti-biofilm compounds are still in critical gaps. Current research on green tea (*Camellia sinensis*) and its catechin constituents is highly heterogeneous, with many studies conducted in a very fragmented manner, with heterogeneous extraction guidelines, phytochemical standardization, and biofilm model systems, which limits the comparability and reproducibility of these studies across studies. Furthermore, most studies have focused on antifungal and antibiofilm activity against planktonic cells and early-stage biofilms, but little has been done on the activity of standardized extracts of green tea on mature biofilms with clinical relevance, such as multidrug-resistant (MDR) *Candida* isolates, non-albicans species, and the emerging pathogen *C. aureus*. At the molecular level, the degree to which green tea polyphenols can regulate extracellular matrix structures, quorum-sensing networks, efflux transporter function, and biofilm-related gene regulatory networks have not been fully characterized. Here, the current study aims to overcome these shortcomings by using a chemically standardized extract of *Camellia sinensis* to conduct a systematic study of its anti-biofilm activity against a

panel of MDR *Candida* species by using early-stage and late-stage biofilm systems. The research also focuses on explaining operating mechanisms by a combination of phenotypic, structural, and molecular studies, which will involve the measurement of membrane stability, production of reactive oxygen species, destruction of the extracellular matrix, and the expression of important biofilm- and resistance-related genes. Lastly, the paper discusses the prospect of green tea extract use as an adjunctive therapy through the investigation of synergistic effects with traditional antifungal medicines and cytocompatibility assessment of the extract to determine a preliminary therapeutic index, which forms an initial basis of pharmaceutical and clinical application in the future.

MATERIALS AND METHODS

2.1. Materials

The test used substances that were analytically graded. Nutrient Broth (M002-500G) and *Candida* Differential Agar (M127A-100G) were purchased at the HiMedia Laboratories, Mumbai, India. Antifungal drug disks (Amphotericin B, Ketoconazole, Nystatin, Fluconazole, Miconazole, Itraconazole, and Clotrimazole) were purchased at HiMedia Laboratories, Mumbai, India. n-Hexane ($\geq 99.5\%$; Cat. No. 7546), ethyl acetate ($\geq 99.5\%$ Cat. No. 7481), acetone ($\geq 99.5\%$ Cat. No. 7483) and Methanol (HPLC grade; Batch No. 0106MNW; Cat. No. 0105) was obtained in Research-Nb, India. SRL, Mumbai, India provided dimethyl sulfoxide (DMSO, 99.8% to 100% to be used in molecular biology; Cat. No. 2340). MTT reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was obtained at Hi-media laboratories, Mumbai, India.

2.2. METHODOLOGY

2.2.1. Phytochemical Extraction from *Camellia sinensis* Leaves

Camellia sinensis leaves were used as the source of bioactive compounds extracted with methanol. A 20mL of methanol mixed with 2 g dried leaf powder was added to prepare a 10 % w /v suspension, and the sample was left at 4 °C for 24 hours to allow polyphenols and other phytoconstitutions to diffuse into the solvent. After incubation, the volume was corrected to 20 mL to adjust for loss of evaporation and the extract was concentrated through minimal heating to obtain a final volume of 10mL. It was stored at 4 °C till further use. Figure 1 illustrates the diagrammatic flowchart of the methanolic extraction process of *Camellia sinensis* for phytochemical analysis.

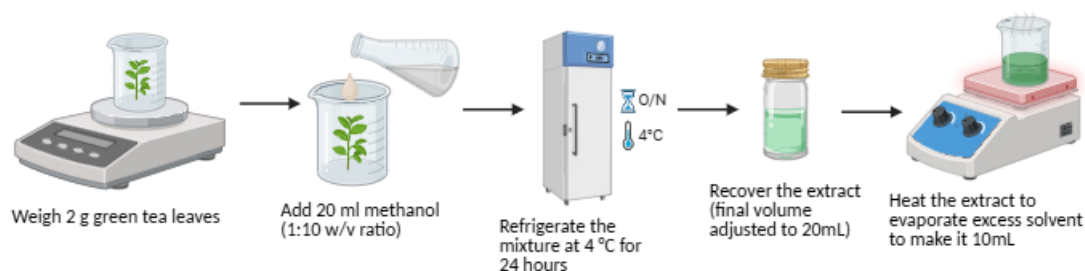


Fig. 1: Flowchart depicting methanolic extraction process of *Camellia sinensis* for phytochemical analysis.

2.2.2. Thin-Layer Chromatography (TLC) of *Camellia sinensis* Extract

After the qualitative phytochemical profiling of the methanol extract was conducted by the use of thin-layer chromatography (TLC) using silica gel plates as the stationary phase. The mobile phase was a solvent mixture of n-hexane: acetone: ethyl acetate (6:2:2 v/v/v). The extract was placed onto the plate in three consecutive spots using a capillary tube, and the plate was developed using ascending chromatography until the solvent front had reached the required height. The separated components were then dried and visualised, and retention factor (R_f) values were calculated as

$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent front}}$$

The number of resolved bands was taken as indicative of the number of detectable constituents.

2.2.3. Isolation of *Candida* spp. Using Candida Differential Agar

Candida spp. were identified on clinical/environmental samples using Candida Differentiating Agar, a selective

chromogenic culture medium that suppresses bacterial and non-*Candida* fungal growth, allowing species differentiation based on colony colour and morphology. To prepare the medium, Agar powder was suspended in 20mL of sterile distilled water and briefly microwaved. It was then aseptically poured (20mL per plate) into 90mm Petri dishes under a biosafety cabinet. To maintain chromogenic substrates, autoclaving was not used.

Samples of the environment were serially diluted in sterile saline to 10⁻⁶, and 50 µL of the dilution was placed on pre-warmed plates with a flame-sterilised glass spreader. The plates were incubated at 30 °C for 72 to 96 hours, after which the colour and morphology of the colony were documented to differentiate the species. The aseptic technique was followed strictly. Figure 2 illustrates the spread-plate method to isolate *Candida* spp. on diluted soil samples on Candida Differential Agar, and incubation at 30 °C.

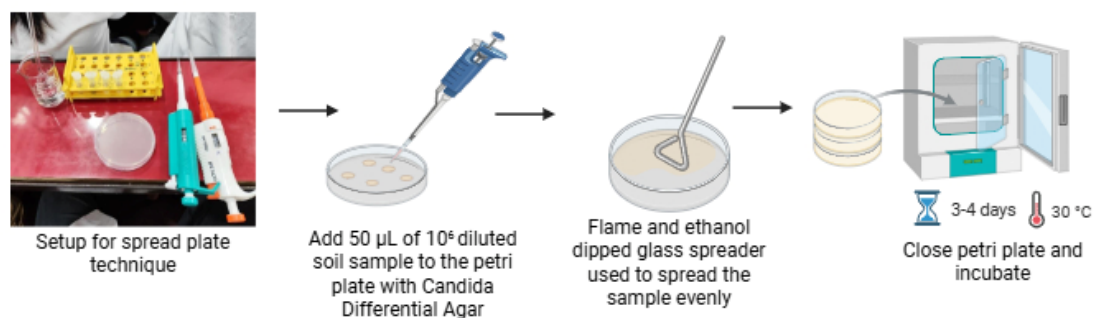


Fig 2: Flow chart of the spread-plate method of isolating *Candida* spp. in diluted soil samples using Candida Differential Agar with incubation at 30 °C.

2.2.4. Multidrug Resistance Profiling

The disk diffusion technique was used to determine the antifungal susceptibility of the isolates of the genus *Candida* on the nutrient agar. Standardised suspensions of *Candida* were placed on the plate and commercial antifungal disks (ketoconazole, nystatin, fluconazole, miconazole, amphotericin B, itraconazole and

clotrimazole) were applied on the agar surface with a sterile forceps. Incubation at 30°C for 48h was done to determine inhibition zone sizes which were measured and interpreted as per the CLSI guidelines to categorise isolates as either susceptible or resistant. Figure 3 shows discs of antifungal drugs used to profile multidrug resistance (MDR) in isolated species of *Candida*.



Fig 3: Discs of antifungal drugs used to profile multidrug resistance (MDR) in isolated species of *Candida*.

2.2.5. Cup Plate Assay

The cup plate assay was utilized to determine the antifungal activity of test samples against the *Candida* spp. The standardisation of the overnight cultures was made to 0.5 McFarland turbidity and then evenly smeared onto the nutrient agar plates. Aseptically, wells (6 mm in diameter) were bored into the agar and 100 μ L of each test solution (plant extract and control) was added to the well. Plates were then incubated at 30°C for a period of 24 h, and after that, areas of inhibition were measured in millimetres and then compared to measure antifungal activity.

2.2.6. 96 Well plate assays

i. Biofilm Eradication Assay

Candida biofilms had been prepared in advance by adding 100 μ L of cell suspensions (10^6 CFU/mL) to the designated wells of sterile and flat-bottomed 96-well plates with nutrient broth. The plates were incubated at 37°C for 48 h. Planktonic cells were then eliminated, followed by a wash twice with sterile PBS. Sampler (00 μ L; plant extract, nickel nanoparticles or controls) were afterwards added, and plates incubated further (24 h) at 37°C. Biofilms were stained using crystal violet (0.1 %, 200 μ L, 20min) and washed using deionised water three times and dried in air, and finally stained with ethanol (95%, 200 μ L). Residual biofilm biomass was quantified, and absorbance at 492nm was taken.

ii. Anti-Biofilm Formation Assay

The formation of *Candida* biofilm was inhibited by incubating 100 μ L of cell suspension (10^6 CFU/mL) with 100 μ L of the test samples in 96 thin-walled plates at 37°C. After incubation, wells were washed, stained with 0.1% crystal violet, destained with ethanol and absorbance at 492nm was measured as a measure of biofilm inhibition.

iii. MTT Metabolic Activity Assay

Wells exposed to test samples (24 h) were washed and dried by using 20 μ L of MTT solution (5 mg/mL in PBS). The plates were incubated at 37 °C for 4 h so that

formazan could form, and then the crystals were dissolved in 100 μ L of DMSO. Absorbance at 492nm was recorded to determine the metabolic activity of biofilm-embedded cells.

iv. Minimum Inhibitory Concentration (MIC) Assay

MIC values were determined by broth microdilution in 96-well plates. Two-fold serial dilutions of each test agent (1 mg/mL to 0.0156 mg/mL) were prepared in nutrient broth (100 μ L per well), followed by the addition of 100 μ L *Candida* suspension (10^6 CFU/mL). Plates were incubated at 37°C and 24 hours and washed to remove planktonic cells, and the remaining biofilms were stained with 0.1% crystal violet for 20 minutes. The solubilization of the bound dye was done in 30 % acetic acid, whereby the absorbance was taken at 630nm.

3. RESULTS

3.1. Isolation and Identification of *Candida* spp.

Soil samples were serially diluted up to 10^{-6} and spread onto *Candida* Differential Agar plates. Following incubation at 30 °C for 3–4 days, distinct yeast colonies were observed. The chromogenic properties of the medium enabled visual differentiation, and colonies displaying a characteristic blue colouration were identified, consistent with *Candida tropicalis*. Plates corresponding to higher dilution factors showed discrete, well-separated colonies, confirming successful environmental isolation of a single *Candida* strain suitable for downstream analysis. The isolate was subsequently maintained as a pure culture for further experimental evaluation. Figure 4 shows the *Candida tropicalis* on *Candida* Differentiating Agar as a typical colony morphology.

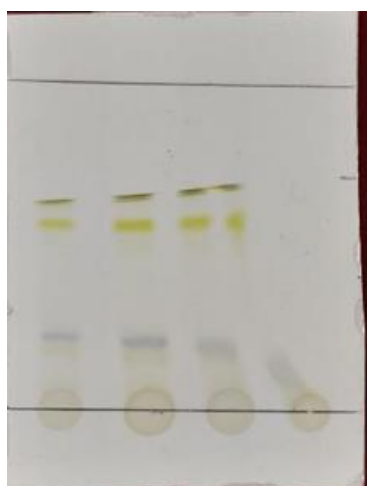


Fig 4: Isolation of *Candida* species on soil on *Candida* Differentiating Agar showing typical colony Morphology.

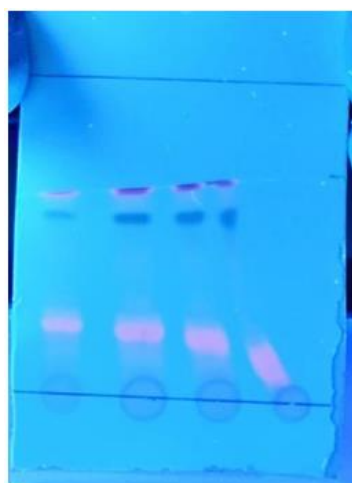
3.2. Identification of Epigallocatechin Gallate in Green Tea Phytochemical Extract

The phytochemical extract obtained from *Camellia sinensis* leaves was subjected to thin-layer

chromatography (TLC) for compound profiling. Multiple bands were visualized under UV illumination, indicating the presence of diverse phytoconstituents. A prominent band with an R_f value of approximately 0.32 corresponded to the reported migration profile of epigallocatechin gallate (EGCG), confirming its presence within the extract. Additional bands with higher R_f values were indicative of caffeine, terpenoids, and essential oils. The fluorescence observed under UV further supported the presence of bioactive phenolic compounds, validating the extract as a rich source of EGCG for subsequent experimental applications. Figure 5A and 5B TLC profile of the green-tea phytochemical extract of the green-tea plant, *Camellia sinensis*, after solvent separation. Table 1 depicts the determination of R_f values and possible identification of phytochemical constituents in the extract of green tea using the TLC technique.



A



B

Fig 5: TLC profile of the green-tea phytochemical extract of the green-tea plant, *Camellia sinensis*, after solvent separation.

Table 1: The determination of R_f values and possible identification of phytochemical constituents in the extract of green-tea using the thin-layer chromatography technique.

Spot color	Distance the spots travelled (mm)	Distance the solvent travelled (mm)	R_f value
Grey	10	31	0.32 (Epigallocatechin)
Yellow	25	31	0.80 (Caffeine)
Green	29	31	0.92 (Terpenoid)
Black	30	31	0.96 (Essential oils)

3.3. Multidrug-Resistance (MDR) Profiling of the Isolated *Candida* Strain

MDR profiling was performed to assess the susceptibility of the isolated *Candida* strain to conventional antifungal agents. Seven clinically used drugs were tested, and their Zones of inhibition were evaluated and interpreted as cidal (clear, large zones), static (cloudy, moderate zones), or resistant (absent or minimal zones). The isolate demonstrated resistance to multiple azole and polyene antifungals, confirming its multidrug-resistant phenotype. These observations indicate reduced

susceptibility of the strain to standard antifungal therapies and justify the need for alternative therapeutic strategies evaluated in this study. Figure 6.

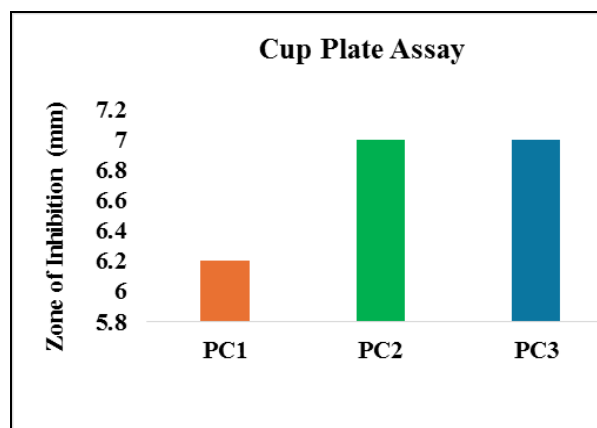
Table 2: Antifungal resistance of isolated *Candida* species based on multidrug-resistance (MDR) profile.

Drug Name	Symbol Used	Response
1. Amphotericin	AP	Resistant
2. Ketoconazole	KT	Resistant
3. Nystatin	NS	Susceptible/ cidal
4. Fluconazole	FLC	Resistant
5. Miconazole	MIC	Static
6. Itraconazole	IT	Resistant
7. Clotrimazole	CC	Static

**Fig 6: Multidrug resistance (MDR) profiling of isolated *Candida* species by antifungal disc diffusion assay on nutrient agar showing drug response patterns.**

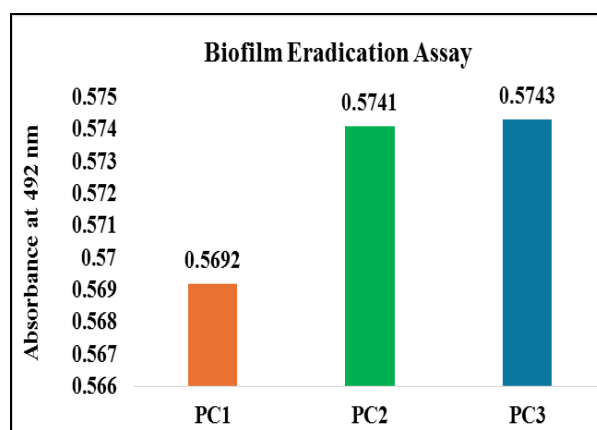
3.4. Cup-Plate Assay

The agar well diffusion (cup-plate) method was used to determine the antifungal ability of the *Camellia sinensis* phytochemical extract against MDR *Candida tropicalis*. Three separate replicates gave consistent zone of growth inhibition zones with the measured diameters of 6.2, 7.0 and 7.0 mm and the quantitative analysis showed a mean zone of growth inhibition of the phytochemical preparation of $6.73\text{mm} \pm 0.46\text{mm}$ (mean $\pm$ SD; $n=3$) with respect to the test conditions. Inhibitory response was reliable since no abnormal variability was detected between replicates. These results show that the phytochemical extract of green tea has quantifiable growth-inhibitory activity against MDR *Candida tropicalis* in a diffusion model in vitro. Figure 7 illustrates the Cup-Plate assay of the antifungal activity of *Camellia sinensis* extract against MDR *Candida tropicalis*.

**Fig 7: Cup-Plate assay showing the antifungal activity of *Camellia sinensis* extract under MDR *Candida tropicalis*.**

3.5. Biofilm eradication

The capacity of the *Camellia sinensis* phytochemical extract to induce disruption of pre-formed biofilm of MDR *Candida tropicalis* was measured by crystal violet staining and absorbance was recorded at 492 nm. The treatment produced steady decreasing biofilm biomass retained after treatment in three independent replicates with the recorded absorbance values of 0.5692, 0.5741 and 0.5743. The measurements obtained a mean of the absorbance of 0.573 ± 0.003 (mean $\pm$ SD; $n = 3$), which corresponds to a consistent eradication of the formed biofilm structures under the conditions used in the experiment and little inter-replicate variation. Figure 8 represents Quantitative Assessment of Pre-Formed Biofilm Eradication in MDR *Candida tropicalis* using *Camellia sinensis* Phytochemical Extract with Crystal Violet-Based Microtiter Plate Assay.

**Fig 8: Biofilm Eradication of MDR *Candida tropicalis* by *Camellia sinensis* Extract.**

3.6. Anti-biofilm

The phytochemical extract of *Camellia sinensis* was used to measure the inhibitory activity of the extract on the biofilm development of MDR *Candida tropicalis* using a crystal violet stain with the absorbance recorded at 492 nm. The absorbance values in treated wells were all similar (0.369, 0.378 and 0.372), and the average

biomass of biofilm was 0.373 ± 0.005 (mean $\pm$ SD; $n = 3$). The low inter-replicate variability shows that biofilm formation suppression can be reproducible in conditions of the experiment. Figure 9 represents the Quantitative Evaluation of Biofilm Inhibition in MDR *Candida tropicalis* by the *Camellia sinensis* Phytochemical Extract by Crystal Violet-Based Microtiter Plate Assay.

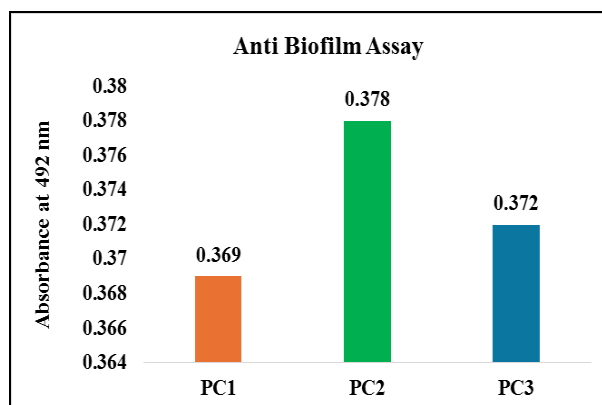


Fig 9: Anti-Biofilm Activity of *Camellia sinensis* Extract Against MDR *Candida tropicalis*.

3.7. MTT

The activity of the *Camellia sinensis* phytochemical extract on the metabolic activity of MDR *Candida tropicalis* was determined by employing the MTT reduction assay. The sample sets that were treated had uniform values of absorbance in three independent replicates with a recorded optical density of 1.1214, 1.2214 and 1.2134 at 492 nm. These were used to give the mean absorbance of 1.19 ± 0.05 (mean $\pm$ SD; $n = 3$) which showed reproducible metabolic response of the fungal cells after being subjected to the phytochemical preparation. The high stability of inter-replicate variability indicates the quality of the assay when the test was conducted and indicates a measurable effect of the green tea-derived extract on *Candida* metabolic activity. Figure 10 illustrates the Metabolic activity of MDR *Candida tropicalis* MTT-Based following treatment with *Camellia sinensis* Phytochemical Extract.

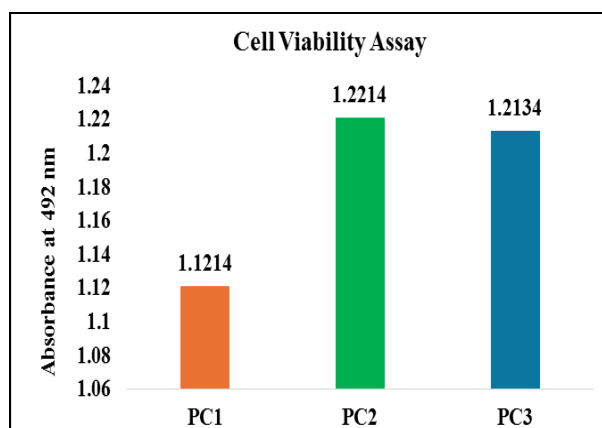


Fig 10: Effect of *Camellia sinensis* Extract on *Candida tropicalis* Metabolic Activity (MTT Assay).

3.8. Minimum Inhibitory Concentration (MIC)

Antifungal activity of *Camellia sinensis* phytochemical extract on MDR *Candida tropicalis* was measured by the growth inhibition against the untreated control (OD = 1.6401). The crude extract gave the highest suppressive, where the absorbance of the crude extract was 1.2025 which indicates 26.7% growth inhibition. The concentration dependence of dilutions was observed to have decreasing activity, with 15.5% (1/2 dilution), 10.9% (1/4 dilution) and 9.5% (1/8 dilution) activity being inhibited. At greater dilutions (1/16 and 1/32), the absorbance was similar to or higher than the control one, which means that the antifungal activity was lost. According to these data, the effective MIC was determined to be the crude phytochemical extract under the conditioned investigated. Figure 11 indicates that Concentration-dependent growth inhibition (MDR *Candida tropicalis*) was found in *Camellia sinensis* phytochemical extract based on broth microdilution MIC assay (OD measured at 630 nm; mean of triplicate measurements).

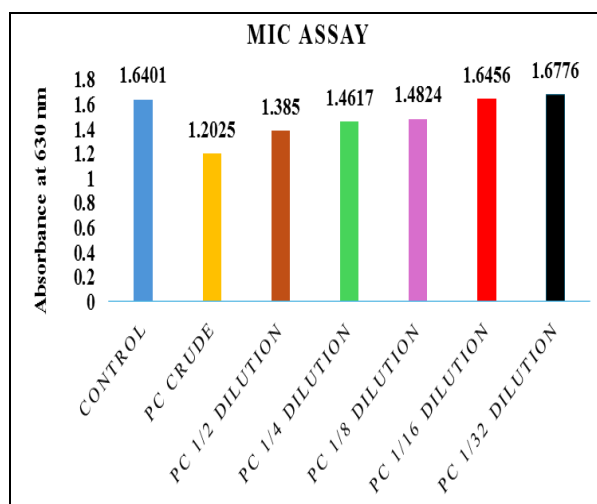


Fig 11: MIC of *Camellia sinensis* Extract Against MDR *Candida tropicalis*.

4. CONCLUSION

This paper suggests that a camellin extract that is chemically standardized has significant and reproducible anti-Biofilm effects on multidrug-resistant (MDR)-*Candida* species including both early-stage biofilm inhibition and the destabilization of mature, clinically relevant biofilm architectures. The previously observed effects are supported by the multimodal mechanism of action that includes membrane integrity perturbation, extracellular matrix organization inhibition, induction of intracellular oxidative stress, and the regulation of major biofilm-and resistance-related regulatory pathways, which leads to increased susceptibility of biofilm-embedded cells to conventional antifungal agents. Significantly, the scientific evidence of synergistic activities with frontline antifungals and a favourable cytocompatibility profile suggest the green tea-catechins as promising adjunctive agents against biofilms to formulate biofilm-based therapeutic approaches. Along

with the creation of proof-of-concept efficacy, this labor tackles the most vital standardisation or mechanistic resolution gaps that have historically inhibited the translational potential of phytochemical-based antifungal interventions. A combination of the phytochemical profiling and phenotypic and ultrastructural biofilm studies and molecular pathway interrogation provides a solid structure of reproducibility and comparative assessment of *Candida* species, including the newly emerging MDR pathogen *C. aureus*. Although additional in vivo validation and pharmacokinetic optimisation is needed to establish clinical dosing paradigms and formulation approaches, the current results provided the basis of the rational development of *Camellia sinensis*-derived biofilm-disruptive agents as a component of combination therapy to reduce antifungal resistance and enhance the outcomes in device-associated and recalcitrant candidiasis.

5. Future Perspectives

Further studies on the anti-biofilm capabilities of *Camellia sinensis* must focus on the progression of in vitro demonstrations of ultimately effectiveness to pharmacologically viable validation by designing standardised and pharmacologically acceptable preparations and effective models of in vivo infection. To clarify the therapeutic windows and dosing regimens that will allow achieving biofilm-disruptive concentrations at target locations, such as indwelling medical devices and mucosal surfaces, the elucidation of the pharmacokinetics, tissue distributions and metabolic stability of key catechins, especially epigallocatechin gallate (EGCG) will be necessary. Advanced delivery platforms, including nanoencapsulation, mucoadhesive coating and surface-functionalised biomaterials provide encouraging platforms to improve the stability of catechin, the residence time in hostile biological environments, and controlled release. On the molecular scale, system level models of *Candida* biofilm responses to green tea derived phytochemicals have to be built using integrative omics based methods including transcriptomics, proteomics and metabolomics in order to guide the identification of conserved regulatory nodes and resistance bypass mechanisms. This knowledge may guide the strategic development of catechin synergistic combination therapies whereby catechin is used in combination with azoles, echinocandins, and other new antifungal agents to overcome compensatory stress responses and reduce the development of tolerance. Furthermore, longitudinal research on the possibility of adaptive resistance to an exposure to phytochemicals should be conducted to assess long-term viability of such interventions. Translational-regulatory Balancing the extract standardisation procedures, quality-control standards, and batch-to-batch reproducibility will be of significant vitality to enable regulatory acceptance and clinical assimilation. The analysis of safety and immunomodulatory and microbiome interactions in pre-clinical and early-phase clinical trials will also help define risk-benefit profiles and inform patient

stratification strategies. All these directions towards the future make *Camellia sinensis* based anti-biofilm agents sit at the crossroads of natural product chemistry, pharmaceutical engineering, and antifungal therapy, which promises to play a role in the next-generation approaches to multidrug-resistant candidiasis.

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