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ANTI-CANDIDA ALBICANS EFFECT OF IMPATIENS WALLERIANA AERIAL EXTRACT

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ABSTRACT: The genus *Impatiens* encompasses a diverse array of species valuable as sources of natural medicine some of which remain unexplored and insufficiently characterized. *Impatiens* is a reservoir of metabolites such as phenolics, phytosterols, triterpenoids, and peptides which exhibit spectrum of activities including antimicrobial, cytotoxic, anti-inflammatory, antioxidant and anti-anaphylactic. *Impatiens walleriana* belonging to the family balsaminaceae is known for its aesthetic appeal and as folk medicine. Drug resistant strains of *Candida* and *Aspergillus* have made fungal infection treatment difficult and plants are serving as good alternatives. *Impatiens walleriana* is anti-microbial and traditionally applied to skin in folk medicine but has not been specifically screened for its anti-*Candida albicans* effectiveness hence, the present *in-vitro* study. The hydroalcoholic extract of the aerea parts of *Impatiens walleriana* has shown anti-*Candida albicans* effect with MIC 3.3mg/ml and ZOI ranging from 9mm to 20mm with maximum effectiveness at 100mg concentration in the triplicate readings taken for the selected doses 50mg, 100mg and 200mg.

INTRODUCTION: The development of invasive fungal infections is a significant health problem particularly in immunocompromised and hospitalized patients. These fungal infections are associated with a high mortality and morbidity rate ². The most commonly identified *Candida* species in hospitals is *Candida albicans*. The rise in antifungal resistance around the world is one of the greatest emerging health problems, making it difficult to select effective antifungal treatments ^{3, 4}. Many strains of *Candida albicans* are resistant to current antifungal drugs, including azoles and echinocandins ⁵.

A significant factor in the development of *Candida albicans* resistance is extended exposure to antifungal drugs, particularly the excessive use of azoles and echinocandins as prophylactics, or the empiric treatment of patients at risk of developing invasive candidiasis. *Candida* species are normally harmless microorganisms that inhabit the oral, gastrointestinal, urinary and vaginal mucosal surfaces of humans ⁶, but they can behave as opportunistic pathogens with the ability to cause both superficial and invasive systemic fungal infections.

The *Candida* genus is, in fact, the most frequently isolated cause of hospital-associated fungal infections, broadly referred to as candidiasis. Although over 150 species are included within this highly diverse genus, only a small number are recognized as significant agents of human disease. One of the key virulence traits of *Candida* is its capacity to survive in multiple environments and to



form surface-adhered microbial structures called biofilms⁷. Fungal diseases also known as mycoses are infection caused by fungi. They can affect various parts of the body ranging from skin and nails to internal organs. *Candida* species are commensal micro-organisms in human oral mucosa, digestive and vaginal tract and spread of this opportunistic fungus happens when host immune system is broken down. These infections can be superficial such as thrush, vaginitis, skin infection or invade the blood stream and spread to any site of human host which cause many clinical complications such as brain abscess, endocarditis, meningitis, arthritis etc⁹.

Medicinal plants have traditionally been used for managing fungal infections, offering a rich source of bioactive compounds for new drug development. Plant-based therapies are gaining popularity as safer, multi-target alternatives to conventional treatments, particularly in fungal infection, which accounts for nearly one-fifth of dermatology visits. Medicinal plants remain essential to global healthcare, particularly in developing regions where traditional medicine is widely practiced. Historically, over 80% of medicines were plant derived, and herbal knowledge has significantly contributed to pharmaceutical development. Their continued use is attributed to perceived safety, effectiveness, affordability, and minimal side effects, yet much of this traditional knowledge often passed down orally is at risk of being lost without documentation^{9,10}.

Medicinal plants such as *Impatiens walleriana* are rich in phytochemicals and hold promise as natural antifungal agents⁶⁻⁸. Among such plants, the genus *Impatiens* is known for its antioxidant, antimicrobial, anti-inflammatory and anti allergic activities. *Impatiens walleriana*, an ornamental species from the Balsaminaceae family used in folk medicine, displays antioxidant, antibacterial and antifungal properties^{6, 5}. Since, this plant is traditionally applied to the skin, easily available, rich in minerals and considered safe. *Impatiens* is extensively distributed across the globe and is easily cultivated in various settings such as home, gardens, green houses and public parks. This research focuses on assessing the aerial extracts of *Impatiens walleriana* for the potential antifungal properties¹⁰.

Impatiens walleriana has not been screened for anti-fungal activity. In the present study extract of this plant has been evaluated for antifungal activity^{10, 11}.

MATERIALS AND METHODS: The fresh plants were first washed under running tap water and root portions were removed followed by drying in shade at room temperature. Dried plant material was ground to coarse powder and stored in an air tight bottle. Extraction was done using 75% hydro alcoholic solvent by maceration for 72 hours. An orbital shaker was used to mix plant material and solvent uniformly during extraction, enhancing the release of active compounds. Solvent was removed by evaporator using water bath and residue was scraped by using spatula and collected followed by weighing and calculation of % yield.

$$\% \text{ Yield} = W_1 / W_2 \times 100$$

Where, W_1 = weight of the extract after extraction.
 W_2 = weight of plant powder.

MIC Test Procedure: 9 dilutions of each drug have to be done with Thioglycolate broth for MIC.

1. In the initial tube 200µl Compound of desired percentage concentration is added into 200µl of TGB (Tube 1).
2. For dilution 200µl of TGB are added in to the next 9 tubes separately (Tube 2 to Tube 10).
3. Then from second tube 200µl was transferred to the next tube containing 200µl of TGB. This was considered as 10-1 dilution.
4. From 10-2 dilution tube 200µl was transferred to the tube 3 (T3) to make 10-2 dilution.
5. The serial dilution was reported up to 10-9 dilution for each drug. As shown in **Fig. 1**.
6. From the maintained stock culture of required organisms 5µl was taken and added into 2ml of TGB.
7. In each serially diluted tube 200µl of above culture suspension was added.
8. The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity.

9. For MIC of Compound antibiotic tetracycline 10mg mixed with 1ml distilled water: 1% Compound prepared and performed the MIC as per the above instructions.

Disc Diffusion Test Procedure:

1. Media used: Brain Heart Infusion agar

2. **Temperature:** Bring agar plates to room temperature before use

3. Inoculum Preparation:

- A. Using a loop or swab, transfer the colonies to the plates.
- B. Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device.

4. Inoculation of Agar Plate:

- A. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum.
- B. Swab entire surface of agar plate three times, rotating plates approximately 60° between streaking to ensure even distribution. Avoid hitting sides of petriplate and creating aerosols.
- C. Allow inoculated plate to stand for at least 3 minutes but no longer than 15 minutes before making wells.

5. **Stock Solution Preparation:** Prepare the stock solution weighing 50mg, 100mg and 200mg of compound and dissolve it in 1ml DMSO

6. Addition of Compound into Plate:

- A. Take hollow tube of 5mm diameter, heat it. Press it on above inoculated agar plate and

remove it immediately by making a well in the plate. Likewise, make 5 well on each plate.

With the help of micropipette add 50µl of each concentration prepared in each well. This will be done for one compound when they want to know the quantity grading. If they want to compare between different compounds then in one plate different compounds will be put with fixed quantity i.e. 50µl. As shown in Fig. 2A, B, C and D.

7. Incubation:

- A. Incubate plates within 15 min of compound application.
- B. Invert plates and stack them no more than five high.
- C. Incubate for 18-24 hours at 37 °C in incubator.

8. Reading plates:

- A. Read plates only if the lawn of growth is confluent or nearly confluent.
- B. Measure diameter of inhibition zone to nearest whole millimeter by holding the measuring device.

RESULTS AND DISCUSSION:

Results: *Impatiens walleriana* extract has resinous consistency and brown appearance and earthy & classy odour. The yield of extract in the present study was found to be 13.75%.

- ❖ Percentage yield of *Impatiens walleriana* extract
- ❖ Weight of dried *Impatiens walleriana* sample: 80gm
- ❖ Weight of *Impatiens walleriana* extract: 11gm
- ❖ Yield of extract was found to be: 13.75 % w/w



FIG. 1: RESULTS OF MIC

TABLE 1: MIC RESULTS

Tube	1	2	3	4	5	6	7	8	9	10	11	12
Constituents	200µl1%Comp+200µlTGB	0µl1%Comp+200µlTGB+200µlOrg	200µl0.33%Comp+200µlTGB+200µlOrg	200µl0.11%Comp+200µlTGB+200µlOrg	10.04%Comp+200µlTGB+200µlOrg	200µl0.013%Comp+200µl1TGB+200µlOrg	200µl0.0433%Comp+200µlITGB+200µlOrg	200µl0.01433%Comp+200µlTGB+200µlOrg	200µl0.004811%Comp+200µlTGB+200µlOrg	200µl0.1603X10 ⁴ %Comp+200µlTGB+200µlOrg	PC200µlTGB+200µlOrg+200µlITR	NC200µlTGB+200µlComp
Compound concentration (in %)	0.5	0.33	0.11	0.04	0.013	0.0433	0.014433	0.004811	0.1603X10 ⁴	0.055X10 ²	50	50
Compound Concentration (inmg/ml)	5	3.3	1.1	0.4	0.13	0.433	0.14433	0.04811	1.603X10 ⁴	0.551X10 ²	5	5
Dilution Factor	Neat	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	-	-
<i>Candida albicans</i>	S	S	R	R	R	R	R	R	R	R	√	√

Note: S=Sensitivity, R=Resistant

Results of Disc Diffusion Method:

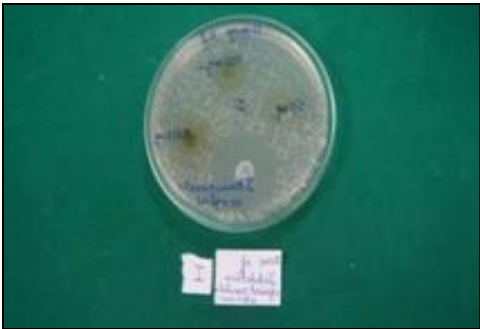


FIG. 2A: ZOI RESULTS (I SET)

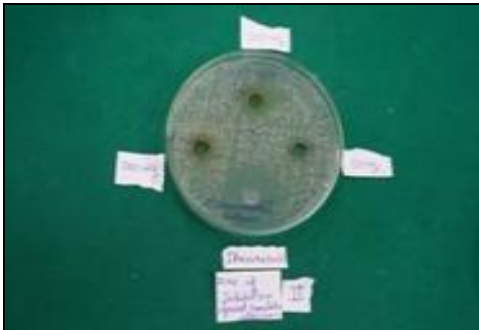


FIG. 2B: ZOI RESULTS (II SET)

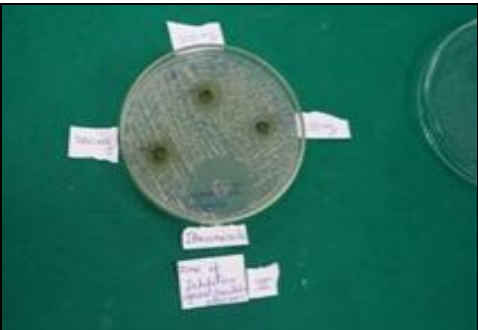


FIG. 2C: ZOI RESULTS (III SET)

TABLE 2: ZONE OF INHIBITION AGAINST *CANDIDA ALBICANS*

Compound concentration (in mg/ml)	50	100	200	Control (Tetracycline)
<i>Candida albicans</i>				
I Set	15mm	20mm	16mm	25mm
II Set	R	18mm	09mm	24mm
III Set	R	17mm	17mm	26mm
Mean±SD	5±8.66	18.33±1.52	14±4.35	25±1.0

Statistical Analysis: All experiments were performed with 3 replicates of each treatment (I Set, II Set, III Set).

Statistical analysis was conducted using Microsoft Excel 2007. Data are expressed as means ±SD.

DISCUSSION: Medicinal plants contain bioactive compounds with strong therapeutic benefits. They are studied as alternatives to antibiotics due to safety and efficacy, and can supplement current antifungal therapy.

The present study evaluated the antifungal activity of *Impatiens walleriana* extract against *Candida albicans* using the agar well diffusion method. Interestingly, the results revealed that lower concentrations of the extract produced larger zones of inhibition compared to higher concentrations, indicating a non-dose-dependent response. In contrast to a typical dose-dependent effect, where increased concentration correlates with enhanced antimicrobial activity, the inhibitory effect of this extract does not follow a strictly proportional relationship^{12, 13}.

Several factors may account for this observation. Minor variations in experimental conditions, such as agar thickness, well dimensions, or inoculum density, could influence diffusion and activity. Additionally, at higher concentrations, certain bioactive compounds may exhibit reduced solubility or instability, thereby diminishing their antifungal efficacy. Interactions among multiple constituents within the extract may also contribute, where some compounds potentially antagonize the activity of others at elevated concentrations^{14, 15}.

These findings underscore the complexity inherent in plant-based extracts, which often comprise a mixture of bioactive molecules with variable effects. While *Impatiens walleriana* demonstrates significant antifungal potential, the lack of a strictly dose-dependent response highlights the necessity for careful optimization of extract concentration. Further studies focusing on the isolation of active compounds and mechanistic investigations are recommended to elucidate the factors influencing the observed activity and to determine the most effective concentration for potential therapeutic applications¹⁵.

The MIC is defined as the lowest concentration of the extract at which the bacteria does not demonstrate visible growth. Results of zone of inhibition are large in lower concentration than the higher concentration. As depicted in **Table 2**. The MIC results 3.3mg/ml for this experiment. Here once the dilution goes on increasing the concentration decreases. First it was 5mg/ml then it comes to 3.3mg/ml. As depicted in **Table 1**. The MIC result for our compound was found to be 3.3mg/ml. Usually, when the concentration of a compound is increased, the Zone of Inhibition

(ZOI) should also increase. But in our case, the opposite has happened it has reduced. For 50 mg, the ZOI was resistant. For 100 mg, it showed 15 mm in the first set. After 100 mg, it showed proper results, but after 200 mg, the ZOI should have increased compared to the previous readings. However, it was reduced instead. This variation may be due to multiple factors

The zone of Inhibition is large in lower concentration than the higher concentration. Paradoxical effect (eagle effect): the eagle effect or paradoxical growth effect refer to the reduced efficacy of an antimicrobial agent at higher concentrations. Usually reported in echinocandins (Eg: caspofungin) and some azoles documents particularly in *Candia Spp* and *Aspergillus Spp*¹⁶.

Precipitation at higher concentration/poor diffusion at higher concentrations, especially above solubility limit, compounds may precipitate, reducing diffusion in agar. This results in smaller or absent inhibition zones despite the high actual dose^{15, 16}. Toxicity to agar or organism environment at very high concentrations, some compounds alter pH, osmolarity, or denature proteins in agar leading to poor organism growth or misinterpreted resistance^{15, 16}. Certain antimicrobials (such as peptides or lipids) may aggregate or undergo concentration-dependent inactivation, reducing their biological activity^{16, 17}. At higher concentrations or over time, some compounds may degrade into by-products that either antagonize antimicrobial activity or interfere with microbial growth¹⁷.

CONCLUSION: The present study revealed that *Impatiens walleriana* extract possesses significant antifungal activity against *Candida albicans*. Interestingly, the inhibitory effect did not follow a strictly dose-dependent pattern, as lower concentrations produced larger zones of inhibition than higher concentrations. Minor variations in experimental conditions, including agar thickness and inoculum density, may also influence the observed activity. These results highlight the inherent complexity of plant-based extracts, which often contain a mixture of compounds with variable effects. The findings emphasize the need for careful optimization of extract concentration to achieve maximal antifungal efficacy. Further investigations focusing on the isolation and characterization of

active constituents are warranted. Additionally, mechanistic studies could provide insight into the factors influencing the extract's activity. Overall, *Impatiens walleriana* demonstrates promising antifungal potential for future therapeutic applications.

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CONFLICT OF INTEREST: Nil

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