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ANTIFUNGAL ACTIVITY OF *WITHANIA SOMNIFERA* CRUDE ETHANOLIC EXTRACT DUNAL

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ABSTRACT

Traditional medicinal herbs are widely used globally and have increasingly captured the interest of scientific and pharmaceutical communities. Recent research highlights their potential therapeutic benefits, particularly their ability to combat drug-resistant infections with their strong antibacterial properties. This has spurred investigations into the active components and processes of these plants, revealing promising avenues for new antimicrobial medicines. With the rise of novel infectious diseases and antibiotic resistance, exploring plants as sources of diverse and potent chemical compounds is critically important. In Ayurveda and other traditional medicine systems, *Withania somnifera* is a significant medicinal herb. Current research shows that the ethanolic extract of *Withania somnifera* is highly efficient against fungal cultures of *A. niger*, *Candida albicans*, *Trichoderma asperellum*, *Candida tropicalis*, and *Candida parapsilosis*. Various concentrations of *Withania somnifera 's* ethanolic extracts (20, 40, 60, 80, and 100 microliters) were applied to these fungal cultures. It was discovered that the 100 **µl** concentration (50 mg/ml) worked best against the fungus cultures. The maximum inhibition zone (measured in millimeters) was found against *A. niger* (21 mm), with *T. asperellum* (17.5 ± 0.5), *C. tropicalis* (17 mm), *C. parapsilosis* (15.5 mm ± 0.70711), and *C. albicans* (14.5 mm± 0.70710678) following closely behind.

Keywords: *Withania somnifera* , *A. niger*, *Candida albicans*, *Trichoderma asperellum*, *Candida tropicalis*, and *Candida parapsilosis*

INTRODUCTION-

Medicinal plants are extensively used in traditional medicine globally. Recently, there has been increased attention from pharmaceutical and scientific communities

towards these plants, supported by numerous publications validating their therapeutic potential. Their antimicrobial properties are especially noteworthy in the fight against drug-resistant pathogens. Some plants have demonstrated effectiveness against resistant organisms, prompting research into their mechanisms and active compounds. The urgent need for new antimicrobial agents, given the rise of new infectious diseases and drug resistance, highlights the importance of exploring plants as a source of diverse and effective chemical compounds.

Withania somnifera , also referred to as winter cherry or Ashwagandha, is a significant kharif crop that is utilized in the ancient medical systems of Ayurveda, Unani, and Siddha. In addition to its anti-inflammatory, immunomodulatory, and antioxidant qualities, *Withania somnifera* also possesses antifungal qualities. Withaferin A, a bioactive component, is responsible for some of its many health advantages **[1]**. *Withania somnifera* is a possible natural antifungal drug because of its ability to destabilize *Sporothrix globosa* yeast cells through the action of phytochemicals such as withanone and withaferin A. The strong antifungal qualities of *Withania somnifera* seed oil target the cell membrane and wall of drug-resistant Candida auris, preventing the formation of biofilms and efficiently eliminating mature biofilms **[2, 3]**. With a minimum inhibitory concentration of less than 1.0 mg/mL, the leaf extract of *Withania somnifera* produced the antifungal chemical withaferin A, which exhibited strong antifungal activity against a variety of Fusarium infections **[4]**. Strong antifungal action against *Fusarium culmorum* and *Rhizoctonia solani* is demonstrated by wood treated with *Withania somnifera* fruit extract, which inhibits their growth by 84.07% and 67.03%, respectively **[5]**. *Trichoderma viride* shows notable growth suppression of 64.3% and 69.5%, respectively, against *Alternaria alternata* and *Sclerotium rolfsii*, which are both targets of *Withania somnifera 's* antifungal activity **[6]**. *Withania somnifera* has the potential to be a natural antifungal agent because both organic and aqueous extracts showed significant antifungal activity against *Fusarium oxysporum* f. sp. *radicis-lycopersici* **[7]**. *Withania somnifera* extract had substantial antifungal activity against *Candida albicans*, demonstrating potential as a therapeutic for fungal infections at inhibitory doses ranging from 50 ppm to 250 ppm **[8]**. Using methanolic and ethanolic extracts, the study assessed the antifungal activity of *Withania somnifera* (Ashwagandha) leaf extracts, demonstrating promise against *Aspergillus niger* **[9]**. *Withania somnifera 's* methanolic root extract shown strong antifungal activity

against *Fusarium oxysporum f. sp. cepae*, reducing fungal biomass by up to 93% **[10]**. Particularly against *Trichophyton violaceum*, *Withania somnifera* extracts demonstrated antifungal activity; the acetone extract proved to be particularly efficient. In the investigation, other extracts failed to exhibit antifungal characteristics **[11]**. *Trichoderma viridae* was susceptible to the antifungal action of *Withania somnifera* extracts, although *Aspergillus species* and *Penicillium chrysogenum* were resistant **[12]**. When *Withania somnifera 's* antifungal properties were assessed against *Aspergillus flavus* and *Candida albicans*, they demonstrated a notable inhibition that was on par with that of the common antifungal medication ketoconazole **[13]**. According to the study, *Withania somnifera* flower extracts shown strong antifungal efficacy against *Aspergillus niger*, on par with the common antifungal medication ketoconazole **[14]**. *Ascochyta rabiei* was susceptible to the antifungal effects of *Withania somnifera*. The ethyl acetate fraction shown total inhibition of fungal biomass, whereas the methanolic extracts of fruit and leaf demonstrated a considerable suppression of fungal biomass **[15]**. Antifungal activity against *Candida albicans*, *Candida tropicalis,* and other fungi was demonstrated by *Withania somnifera*. Significant suppression was seen in ethanol and methanol extracts, with methanol being more efficient against *Candida albicans* **[16]**. Strong antifungal activity of *Withania somnifera* was demonstrated, especially against *Candida albicans*, indicating its potential as a source for antifungal medications in the future **[17]**. In order to effectively battle fungal diseases, *Withania somnifera* demonstrated antifungal action against phytopathogenic fungi, with a 54.44–78.88% decrease of mycelial growth **[18]**. In the study, *Withania somnifera* (WS) demonstrates potential antifungal characteristics by demonstrating antimicrobial activity against *Bacillus subtilis* and *Serratia marcescens* **[19]**. By preventing spore germination and hyphal growth in fungi such as *Aspergillus flavus* and *Fusarium species*, *Withania somnifera* glycoprotein (WSG) demonstrates strong antifungal activities **[20]**.

Pulmonary Aspergillosis may be brought on by *A. niger*. Hemostasis and a persistent, productive cough are signs of pulmonary Aspergillosis. Invasive Aspergillosis can occur in certain cancer patients receiving chemotherapy because of weakened immune systems. In these patients, the infection spreads quickly from the lungs to the brain, heart, kidney, or skin. Asthma, allergy infections, and lung infections can all be brought on by *Trichoderma species*. Organs including bones, the brain and central nervous system, the eyes, heart valves, kidneys, liver, and spleen may be

impacted by *Candida species*. More virulent in nature, *C. tropicalis* has a tendency to infect submucosal blood vessels. Individuals with unbroken immunity typically experience inflammation at the site of infection, which restricts the ability of germs to penetrate. Among these, acute candidal esophagitis is the most prevalent. *Candida albicans* can overgrow in the esophagus or in the mouth and throat (oral thrush). Hospitalized patients who have *Candida* infection of internal organs such as the kidney, brain, or bloodstream develop invasive candidiasis. Neonates, transplant recipients, and patients undergoing parenteral feeding are the main populations affected by *C. parapsilosis*. *Candida parapsilosis* is the second most prolific biofilm-producing species among the *Candida species*, while having a lower mortality rate (4%) than *Candida albicans* **[21, 22]**.

Material and Methods- Sabouraud dextrose agar –SDA (SRL Chem, Cat no.-19427) plates, Fungal Cultures- *A. niger,* (MTCC281), *C. albicans* (MTCC 854), *T. asperellum* (MTCC4347), *C. tropicalis* (MTCC 230), *C. parapsilosis* (MTCC 230), Procured from Microbial Type Culture Collection and Gene Bank (MTCC)-Chandigarh, Solvent (vehicle control)-Dimethyl Sulfoxide- DMSO (SRL Chem 28580), Amphotericin B- (Amphocare)- 5 mg/ml, Amount Loaded – (0 to 5000µg/disc)

Anti-microbial activity assay

The antifungal activity was checked by well diffusion method **[23]**. The SDA plates were inoculated with 100 µl of fungal culture, *A. niger* (MTCC281), *C. albicans* (MTCC 854), *T. asperellum* (MTCC4347), *C. tropicalis* (MTCC 230), and *C. parapsilosis* (MTCC 230). The inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X 108 CFU/mL from Sabouraud dextrosebroth). Next, the wells containing 10 µl of different concentration (0 to 500 mg/ml) were made. One well in each plate was loaded with solvent alone, serving as the vehicle control, and Amphotericin B well (50µg) was taken as the positive control. The plates of *A. niger, C. albicans, T. asperellum, C. tropicalis,* and *C. parapsilosis* were incubated (Basil Scientific Corp. India- Incubator) at 37 °C for 24 hours. The clear zones created around the well were measured and recorded.

Results and discussion- Results indicates significant anti-fungal activity of *Withania somnifera* against these *A. niger*, *C. albicans*, *T. asperellum*, *C. tropicalis* and *C. parapsilosis* fungal cultures. Various concentrations of *Withania somnifera 's* ethanolic extracts (20, 40, 60, 80, and 100 microliters) were applied to these fungal cultures. It was discovered that the 100 **µl** concentration (50 mg/ml) worked best against the fungus cultures. There is no zone of inhibition against fungal cultures at

concentrations of 20 and 40 microliters. A zone of inhibition was seen at 60 **µl** maximum against *A. niger* (14 mm), *C. tropicalis* (13 mm), and *C. albicans* (11 mm). However, no zone of inhibition was seen against *C. parapsilosis* and *T. asperellum*. *A. niger* (16 mm ± 0.70710678) was the first to exhibit a maximal zone of inhibition at 80 **µl**, followed by *C. tropicalis* (14 mm), *T. asperellum* (13.5 mm ± 0.5), *C. parapsilosis* (13 mm), and *C. albicans* (12.5 mm). The maximum inhibition zone (measured in millimeters) was found against *A. niger* (21 mm), with *T. asperellum* (17.5 ± 0.5), *C. tropicalis* (17 mm), *C. parapsilosis* (15.5 mm ± 0.70711), and *C. albicans* (14.5 mm± 0.70710678) following closely behind **(Figure 1 and Table 1)**. According to the study, offers a more effective and viable substitute for common antifungal medications.

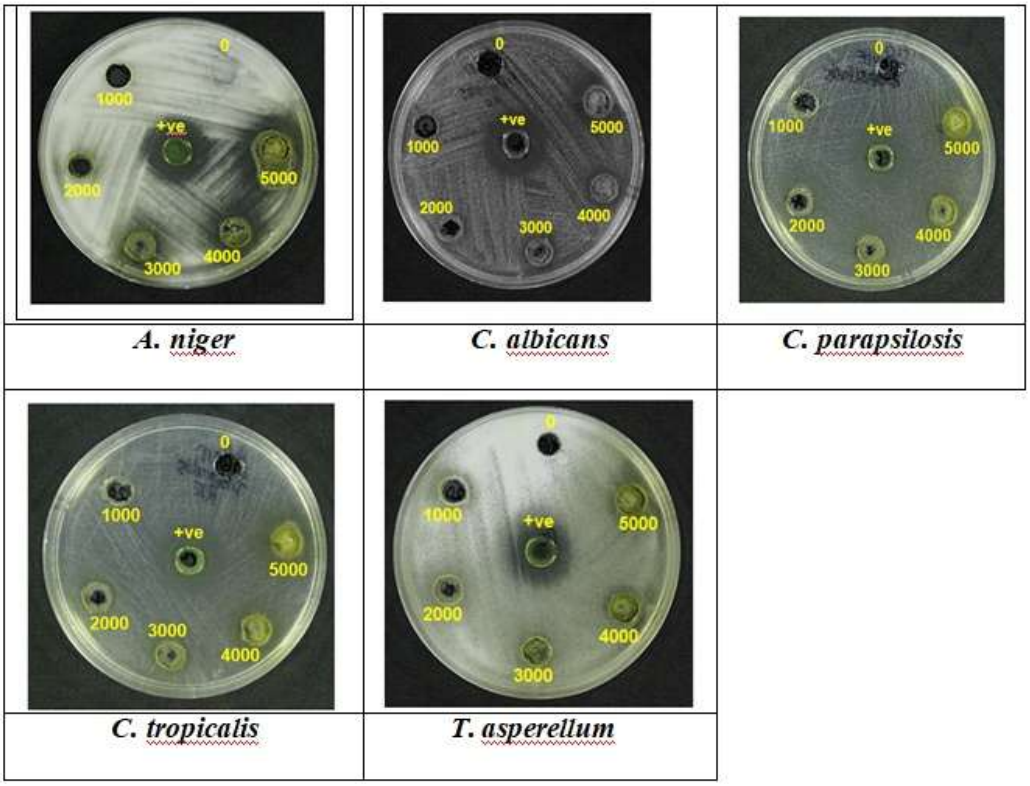
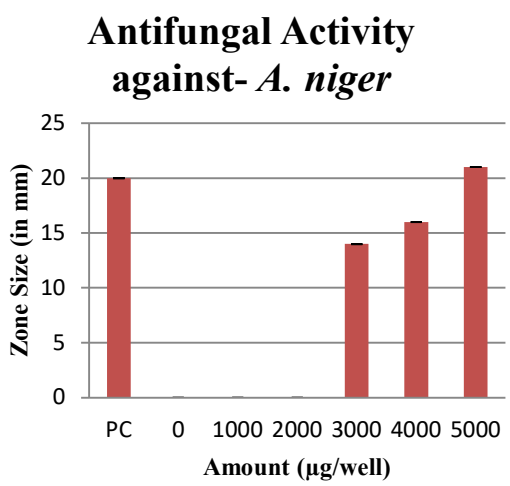


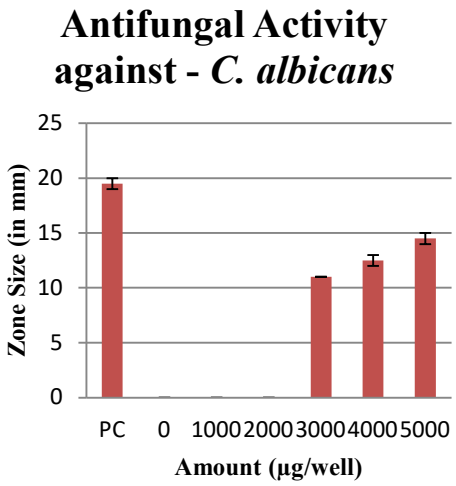
Figure 1: Antifungal activity of Ethanolic whole plant extract of *Withania somnifera* against *A. niger*. *C. albicans*. *C. parapsilosis, C. tropicalis, T. asperellum*

Table 1: Shows the Zone of Inhibition activity (in millimeters) and the antifungal activity of *Withania somnifera* against different cultures

Amount (µg/well)	*A. niger*	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*T. asperellum*
PC	20	19.5 ± 0.70710678	17	19	18.5 ±0.5
0	0	0	0	0	0
1000	0	0	0	0	0
2000	0	0	0	0	0
3000	14	11	0	13	0
4000	16	12.5 ± 0.70710678	13	14	13.5±0.5
5000	21	14.5 ± 0.70710678	15.5 ± 0.70711	17	17.5± 0.5



(A)



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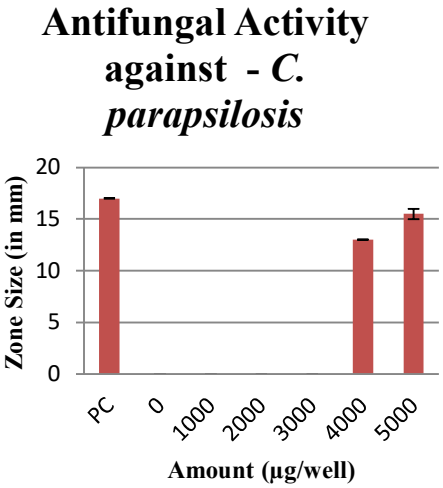
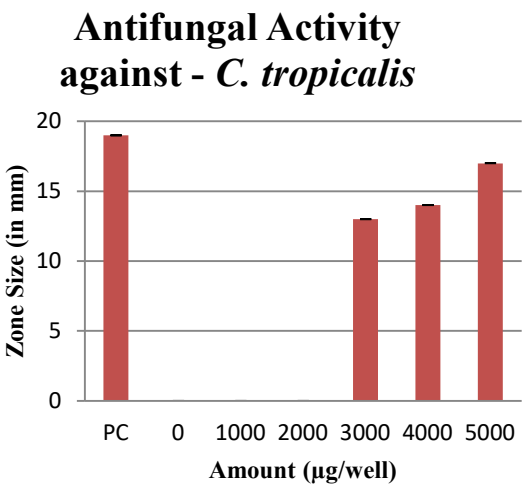
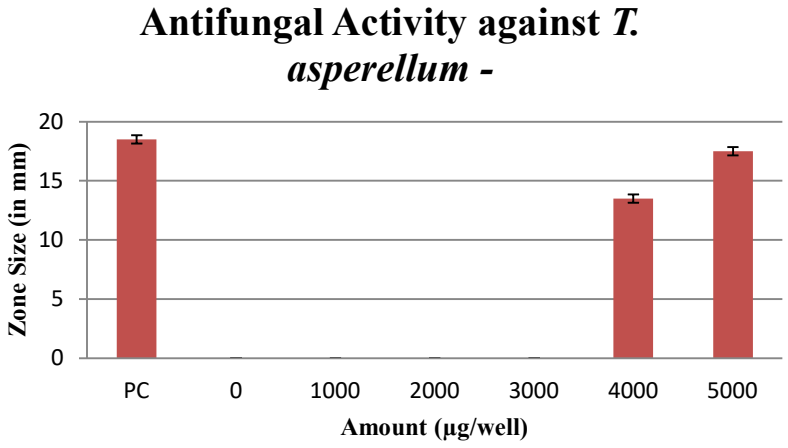


(C)



(D)



(E)

Graph- Graphical representation Antifungal Activity of *Withania somnifera* ethanolic whole plant extract against - (A) *A. niger*. (B) *C. albicans*. (C) *C. parapsilosis*. (D) *C. tropicalis*. (E) *T. asperellum*.

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