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Screening of Fungal Pathogens Isolated from the Oral Cavity and their Response to Plant-based Antifungal Activity

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Abstract: Fungal infections of the oral cavity are a significant health concern, particularly among immunocompromised individuals, due to their potential to cause systemic diseases. This study evaluates the antifungal activity of ethanol, methanol, acteone, and ethanol + methanol extracts from two medicinal plants, *Simaruba glauca* and *Eucalyptus*, against common oral fungal pathogens. A total of 30 oral cavity samples were collected from patients, with 25 testing positive and 5 testing negative for fungal pathogens. The infection were more prevalent in males (22 cases) than females (8 cases). The frequency of isolated oral pathogens was *Aspergillus spp* (0.43%), *Rhizopus spp* (0.13%), *Candida spp* (0.26%). The fungal isolates were characterized and identified through established microbiological methods, and their susceptibility to plant extracts was assessed using disk diffusion and well diffusion method. The results demonstrated significant antifungal activity of the plant extracts, highlighting their potential as effective and natural alternatives to conventional antifungal treatments. This study concludes that *Simaruba glauca* and *Eucalyptus* extracts may serve as promising therapeutic agents for developing novel antifungal treatments, offering a sustainable approach to managing oral fungal infections and reducing the burden of antifungal resistance.

Keyword:-*Oral Fungal Infection, Antifungal Activity,Medicinal Plant, Antifungal Resistance, Plant Based Mouthwash.*

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I. INTRODUCTION

Oral fungal infections are emerging as a significant public health concern, particularly in individuals with compromised immune systems, diabetes, prolonged antibiotic use, or poor oral hygiene. The human oral cavity, a complex ecosystem, harbors diverse microorganisms, including fungal species such as *Candida albicans, Candida glabrata, Candida tropicalis, Aspergillus niger, and Rhizopus stolonifer*. These fungi, under certain conditions, can shift from being commensals to opportunistic pathogens, leading to infections such as oral candidiasis, denture stomatitis, angular cheilitis, and, in severe cases, invasive mucormycosis. Such infections are not only uncomfortable but can also act as reservoirs for systemic dissemination, causing potentially life-threatening conditions. Fungal pathogens within the oral cavity frequently coexist and interact with bacterial species, forming mixed biofilms that exacerbate oral diseases such as dental caries and periodontal disease. For instance, *Candida albicans* synergizes with Streptococcus mutans, enhancing biofilm pathogenicity, which contributes to tooth decay and gum inflammation. Additionally, species like *Aspergillus niger* and *Rhizopus stolonifer* are implicated in invasive fungal infections, particularly in immune compromised individuals. This correlation between fungal and bacterial pathogens complicates treatment and underscores the need for multifaceted therapeutic strategies targeting both types of microorganisms. The rising prevalence of antifungal resistance has further complicated the management of oral fungal infections. Conventional antifungal agents, such as azoles and polyenes, are becoming less effective due to resistance mechanisms, including efflux pump over expression and mutations in fungal enzymes. This escalating problem has driven interest in alternative treatments, particularly those derived from medicinal plants. Medicinal plants such as *Simaruba glauca* and Eucalyptus are gaining attention for their antifungal and antibacterial properties.

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These plants contain bioactive compounds quassinoids in *Simaruba glauca* and eucalyptol in Eucalyptus that disrupt fungal cell membranes, inhibit biofilm formation, and interfere with the growth and survival of pathogens. Plant-based mouthwashes formulated with extracts from *Simaruba glauca* and *Eucalyptus globulus* offer a promising alternative to synthetic drugs. Such formulations can target fungal pathogens, help maintain a balanced oral microbiome, and reduce the recurrence of infections. Moreover, plant-based treatments are generally safer, more cost-effective, and accessible, making them an attractive option for communities with limited healthcare resources. This study evaluates the antifungal efficacy of *Simaruba glauca* and *Eucalyptus* against oral fungal pathogens using standardized disk diffusion and well diffusion methods. It also explores their application in plant-based mouthwash formulations to address the growing challenges posed by antifungal resistance and mixed microbial infections. By leveraging the therapeutic potential of medicinal plants, this research contributes to the development of natural, sustainable solutions for oral healthcare and the management of fungal infections

II. MATERIALS AND METHODS

➢ *Collection of Samples*

A total of 30 oral cavity samples were collected from individuals presenting symptoms of oral fungal infections. Samples were obtained using sterile cotton swabs and stored in sterile transport media. Samples were inoculated on Sabouraud dextrose agar media supplemented with chloromphenicol (0.005mg). plates were incubated at 28º C for 3 to 5 day fungal growth. Individual colonies were isolated and transferred to SDA slant for pure culture.

➢ *Preparation of Medicinal Plant Extracts*

Leaves of *Simaruba glauca* and *Eucalyptus* were collected, washed thoroughly with distilled water, and air-dried under shade to preserve their bioactive compounds. The dried leaves were ground into a fine powder using a mechanical grinder.

➢ *Solvent Extraction*

The powdered plant material was subjected to extraction using four solvents: ethanol, methanol, acetone, and a methanol-ethanol mixture (1:1 ratio). For each extraction: 20 gm of plant powder was mixed with 100 mL of the respective solvent. The mixture was incubated at room temperature for 72 hours with intermittent shaking and extract was filtered using filter paper and concentrated using a rotary evaporator under reduced pressure. The concentrated extracts were stored at 4°C in sterile containers until further use.

➢ *Antifungal Activity Assay*

The antifungal activity of the plant extracts was assessed against the identified fungal pathogens using the disk diffusion and well diffusion methods. for disk diffusion method sterile discs (10 mm) were impregnated with plant extracts .The discs were placed on SDA plates previously inoculated with fungal suspension .Plates were incubated at 28 ^{0}C for 24to 48 hour. and the diameter of the zone of inhibition was measured in millimetres. and for well diffusion method wells of 10 mm diameter were punched into SDA plates inoculated with fungal suspensions. Each well was filled with 100 µL of the plant extract at varying concentrations. The plates were incubated at 28^0 C.

III. RESULTS

➢ *Isolation and Identification of Fungal Pathogen*

Out of the 30 oral cavity samples collected ,25 samples were positive for fungal growth, while 5 samples were negative. Among the positive case, 22(male) and 8 (female). The age distribution analysis that fungal infection were most frequent in individuals aged 0-10 year (7 case), 21-30 year (6 case), 41-50 year (6 case), while fewer cases were recorded in older adults 51-60 year (1 case) above 60 year (1 case). The infection duration ranged from 2 to 11 month, indicating prolonged presence. This distribution indicates that fungal infection are more common in children ,young adults and middle-aged individuals, potentially due to difference in immune response, oral hygiene, and environmental exposure. The fungal species identified were *Aspergillus spp* (0.43%), *Candida spp* (0.26%), and *Rhizopus spp* (0.13%).

Table 1 Detection of A Fungal Pathogen in Oral Cavity Sample.

CASE NO.	AGE/SEX	DURATION	SOURCE	ISOLATED SPECIES
CASE 1	6 Y/M	3 Month	Oral	Candida spp.
CASE 2	8 Y/M	5 Month	Oral	Candida spp.
CASE 3	7 Y/M	3 Month	Oral	-ve
CASE 4	29 Y/F	1 year	Oral	Rhizopus spp.
CASE 5	15 Y/M	7 Month	Oral	Aspergillus niger
CASE 6	45 Y/F	9 Month	Oral	Aspergillus fumigatus
CASE 7	21 Y/F	10 Month	Oral	Aspergillus flavus
CASE 8	18 Y /M	8 Month	Oral	Rhizopus spp.
CASE 9	19 Y/M	9 Month	Oral	Aspergillus oryzae
CASE 10	35 Y/M	1 year	Oral	Aspergillus nidulans
CASE 11	25 Y/M	11 Month	Oral	-ve
CASE 12	10 Y/M	3 Month	Oral	Candida spp.
CASE 13	6Y/M	4 Month	Oral	Candida spp.
CASE 14	12 Y/M	8 Month	Oral	Aspergillus niger
CASE 15	39 Y/M	1 year	Oral	Aspergillus oryzae
CASE 16	61 Y/M	2 year	Oral	Candida spp

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CASE 17	45 Y/M	1 year	Oral	Rhizopus spp.
CASE 18	26 Y/M	3 Month	Oral	Aspergillus flavus
CASE 19	5 Y/M	4 Month	Oral	Candida spp.
CASE 20	3 Y/M	2 Month	Oral	-ve
CASE 21	31 Y/F	1 year	Oral	Aspergillus niger
CASE 22	36 Y/M	1 year	Oral	-ve
CASE 23	44 Y/M	1 year	Oral	Rhizopus spp.
CASE 24	14 Y/F	8 Month	Oral	Aspergillus nidulans
CASE 25	32 Y/M	8 Month	Oral	Aspergillus flavus
CASE 26	37 Y/F	9 Month	Oral	-ve
CASE 27	9 Y/M	4 Month	Oral	Candida spp.
CASE 28	27 Y/F	6 Month	Oral	Aspergillus fumigatus
CASE 29	7 Y/F	4 Month	Oral	Candida spp.
CASE 30	50 Y/M	1 year	Oral	Aspergillus niger

Table 2 Antifungal Activity of Medicinal Plant -the Antifungal Activity of the Plant Extract (Simaruba Gluca and Eucalyptus Was Evaluated Using Disk Diffusion and Well Diffusion Methods).

S.NO.	**ISOLATION**	**SOURCE**	**EXTRACTS OF LEAF**	**DIAMETER**	***SIMAROUBA GLAUCA***		***EUCALYPTUS***	
					ZONE OF INHIBITION			
					DISK	WELL	DISK	WELL
1)	*Candida*	Oral	Ethanol extract	10mm	18mm	19mm	22mm	18mm
		Oral	Methanol extract	10mm	15mm	20mm	16mm	19mm
		Oral	Acetone extract	10mm	10mm	21mm	16mm	17mm
		Oral	Methanol + Ethanol extract	10mm	12mm	19mm	22mm	20mm
2)	*Aspergillus Flavus*	Oral	Ethanol extract	10mm	16mm	13mm	17mm	13mm
		Oral	Methanal extract	10mm	13mm	16mm	16mm	14mm
		Oral	Acetone extract	10mm	No zone	15mm	28mm	11mm
		Oral	Methanol + Ethanol extract	10mm	16mm	19mm	19mm	19mm
3)	*Aspergillus nidulans*	Oral	Ethanol extract	10mm	17mm	13mm	15mm	14mm
		Oral	Methanol extract	10mm	19mm	11mm	16mm	12mm
		Oral	Acetone extract	10mm	20mm	10mm	14mm	11mm
		Oral	Methanol + Ethanol extract	10mm	21mm	16mm	18mm	16mm
4)	*Aspergillus oryzae*	Oral	Ethanol extract	10mm	16mm	14mm	16mm	12mm
		Oral	Methanol extract	10mm	20mm	12mm	20mm	16mm
		Oral	Acetone extract	10mm	No zone	11mm	No zone	No zone
		Oral	Methanol + Ethanol extract	10mm	19mm	14mm	19mm	14mm
5)	*Aspergillus fumigatus*	Oral	Ethanol extract	10mm	30mm	13mm	28mm	15mm
		Oral	Methanol extract	10mm	28mm	14mm	30mm	13mm
		Oral	Acetone extract	10mm	25mm	10mm	26mm	11mm
		Oral	Methanol + Ethanol extract	10mm	29mm	17mm	27mm	14mm
6)	*Aspergillus niger*	Oral	Ethanol extract	10mm	20mm	29mm	17mm	12mm
		Oral	Methanol extract	10mm	22mm	30mm	18mm	13mm
		Oral	Acetone extract	10mm	19mm	No zone	20mm	10mm
		Oral	Methanol + Ethanol extract	10mm	23mm	28mm	19mm	12mm
7)	*Rhizopus*	Oral	Ethanol extract	10mm	29mm	30mm	20mm	30mm
		Oral	Methanol extract	10mm	25mm	26mm	25mm	25mm
		Oral	Acetone extract	10mm	20mm	20mm	21mm	15mm
		Oral	Methanol + Ethanol extract	10mm	26mm	30mm	24mm	17mm

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IV. DISCUSSION

This study assessed the antifungal activity of leaf extracts from Simarouba glauca and Eucalyptus against oral fungal pathogens (Candida spp, Aspergillus spp, and Rhizopus spp). The results revealed significant antifungal potential, with methanol andethanolextracts showing superior activity in both the disk and well diffusion methods. For Simarouba glauca, the methanol extract exhibited the highest zone of inhibition against Aspergillus fumigatus (30 mm in well diffusion), followed by ethanol and methanol-ethanol extracts, indicating the effectiveness of these solvents in extracting active compounds. In contrast, the acetone extract demonstrated the least activity, particularly against Aspergillus flavus. Similarly, Eucalyptus methanol extracts exhibited robust antifungal activity, with maximum inhibition observed against Aspergillus fumigatus (28 mm in well diffusion). Notably, the acetone extract showed no inhibition against Aspergillus oryzae in disc diffusion, underscoring solvent-specific variations in bioactive compound extraction.

The superior antifungal activity of methanol and ethanol extracts can be linked to their ability to extract a broader spectrum of secondary metabolites, including phenolics, flavonoids, alkaloids, and terpenoids. These compounds are well-documented for their antifungal mechanisms, which include disrupting fungal cell membranes, inhibiting ergosterol synthesis, and generating reactive oxygen species. Studies have shown that Eucalyptus leaves contain compounds like 1,8-cineole and tannins, which possess strong antifungal properties (Sartorelli et al., 2007). Similarly, quassinoids and alkaloids from *Simarouba glauca* are known to inhibit fungal growth by targeting cell division and energy metabolism (Seidl et al., 2010).

These findings are particularly relevant in light of the increasing prevalence of antifungal resistance, which limits the efficacy of commonly used drugs such as azoles and polyenes. Resistance mechanisms, including efflux pump overexpression and biofilm formation, complicate the treatment of oral fungal infections. In this context, plant-based antifungal agents provide a promising alternative due to their broad-spectrum activity and low resistance potential. Moreover, synthetic oral care products like mouthwashes and toothpaste often contain chemicals like chlorhexidine and triclosan, which can lead to side effects such as mucosal irritation, discolouration, and microbial imbalance. The natural extracts tested in this study could serve as safer, eco-friendly alternatives, combining antifungal efficacy with minimal side effects. The development of plant-based oral care products could contribute significantly to combating fungal infections while promoting sustainable healthcare practices.

V. CONCLUSION

The findings of this study demonstrate the significant antifungal potential of *Simarouba glauca* and *Eucalyptus* leaf extracts against a range of oral fungal pathogens, including *Candida spp, Aspergillus spp, and Rhizopus spp.* Among the solvents tested, methanol and ethanol extracts exhibited the highest antifungal activity. These results hold significant implications for human health. The use of plantbased antifungal agents offers a natural and safer alternative to synthetic drugs, which are often associated with adverse side effects and contribute to the growing issue of antifungal resistance. Incorporating *Simarouba glauca* and *Eucalyptus* extracts into everyday oral care products, such as mouthwashes and toothpaste, could help prevent and treat oral fungal infections effectively while promoting oral hygiene.

The observed activity of these extracts is likely due to the presence of secondary metabolites, such as phenolics, flavonoids, and alkaloids, which possess antifungal, anti-inflammatory, and antioxidant properties. These compounds not only combat fungal infections but also support the overall health of the oral microbiome, which plays a crucial role in maintaining systemic health. By leveraging these plant extracts, it is possible to address the limitations of synthetic oral care products and develop eco-friendly and cost-effective solutions for oral healthcare. Further studies are recommended to isolate and characterize the active compounds, evaluate their synergistic interactions, and explore their therapeutic applications in clinical settings. This research underscores the potential of integrating natural products into modern medicine to enhance human well-being, offering a sustainable approach to combating oral fungal infections and improving overall oral health.

Fig 1 Macroscopic Identification of Candida Spp

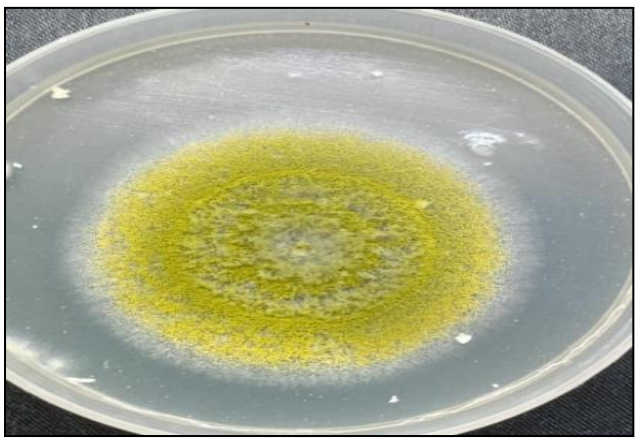

Fig 2 Macroscopic Identification of Aspergillus Flavus

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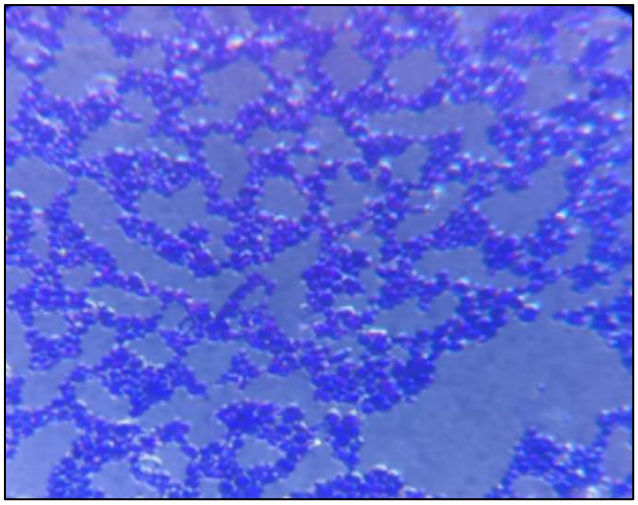

Fig-3 Microscopy Identification of Cotton Blue Staining of Candida Sp

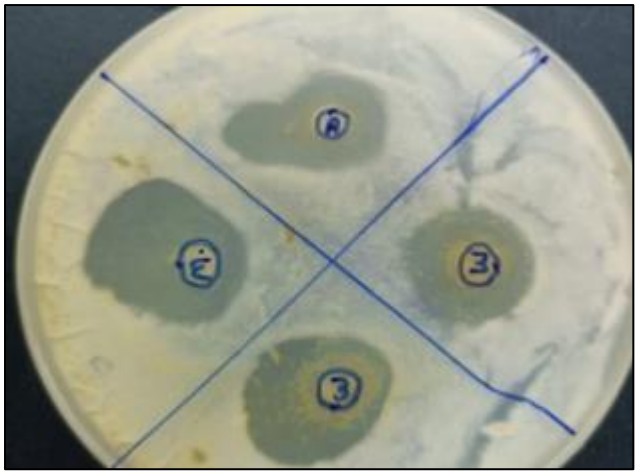

Fig 6 Antifungal Well Diffusion Method Against Candida Spp. from Showing Zone of Inhibition

Fig 4 Microscopy Identification of Indian Ink of Aspergillus Flavus

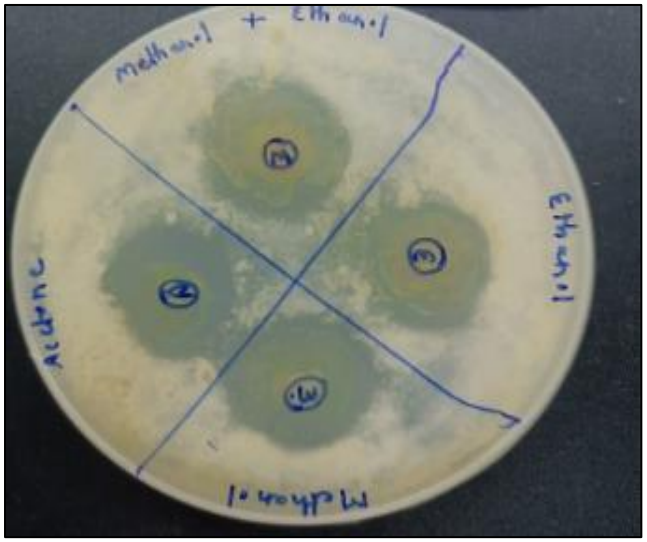

Fig 7 Antifungal Well Diffusion Method Against Aspergillus Flavus from Showing Zone of Inhibition

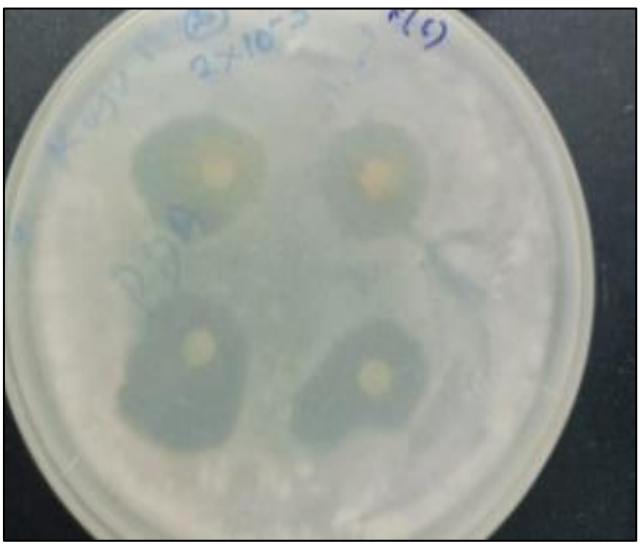

Fig5 Antifungal Disk Diffusion Method Against Candida Spp.from Showing Zone of Inhibition

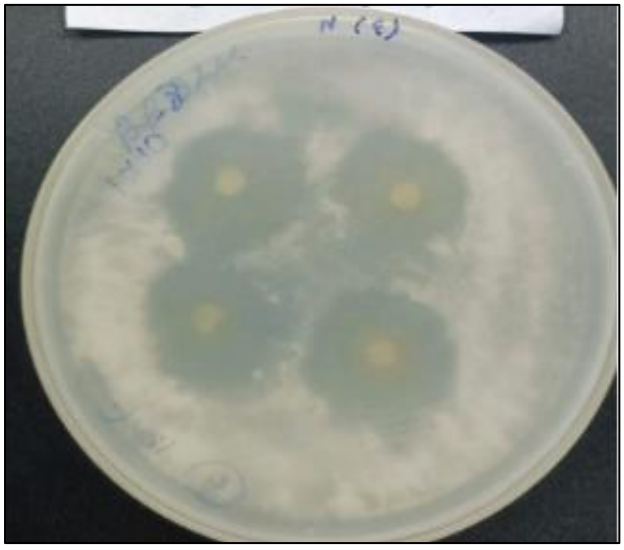

Fig 8 Antifungal Disk Diffusion Method Against Aspergillus Flavus from Showing Zone of Inhibition

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