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COMPARATIVE ANALYSIS OF THE ANTIMICROBIAL ACTIVITY OF *OCIMUM SANCTUM*, *CAMELLIA SINENSIS* & *AZADIRACHTA INDICA* AGAINST *CANDIDA ALBICANS* & *ALTERNARIA*

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Abstract: Dental caries and periodontitis are prevalent and persistent oral diseases posing significant health challenges. The inclusion of fungi as commensals within oral biofilms presents a novel avenue of research in oral biology. This study reviews existing literature on biofilms, with a focus on methodologies, highlighting the coexistence of *Candida albicans* with bacteria in oral biofilms and its implications for oral health. The study explores the efficacy of natural products, particularly traditional herbal remedies like Tulsi, Green Tea, and Neem, as antifungal agents against drug-resistant pathogens. The current study was conducted on *Alternaria* isolated from patients suffering with dental caries and standard strain of *Candida albicans* MTCC 227, procured from IMTECH, Chandigarh. To evaluate the efficacy of these plants against oral biofilm-forming bacteria, phytochemical screening using the methanolic leaf extracts and the antimicrobial activity was checked against the isolated strains. Phytochemical screening revealed the presence of several bioactive compounds in the extracts, with Eugenol from *Ocimum sanctum*, Catechin from *Camellia sinensis* and Nimbidin from *Azadirachta indica*. This study proposes an investigation into the potential of organic herbs to halt or inhibit dental caries and associated diseases, providing a sustainable and alternative approach to oral health management.

Keywords: Biofilm, *Candida albicans*, *Alternaria,* Oral health, Green tea, Neem, Tulsi, Antifungal agents, Thin-layer chromatography

I. INTRODUCTION:

Biofilms are delineated as elaborately structured microbial communities exhibiting adhesion to surfaces, interwoven with their self-synthesized extracellular polymeric substance. Inadequate oral hygiene, dietary habits rich in sugars and starches, individual predispositions, and environmental factors collectively influence biofilm development. Medical conditions, tobacco use further exacerbate susceptibility to biofilm-related oral diseases. The significance of *Candida albicans* within dental biofilms lies in its multifaceted role as an opportunistic pathogen with the potential to cause oral infections and influence the persistence of endodontic infections, thereby impacting the outcomes of endodontic treatments. *Candida albicans* is the most common species of Candida recovered from the oral cavity, and it is estimated to account for over 80% of all oral yeast isolates. Its prevalence is further highlighted by the fact that it is often present in both the commensal state and in cases of oral candidiasis. (Janus *et al.,* 2017). While *Alternaria* is more commonly associated with plant pathogens and environmental allergens, recent studies have begun to shed light on its presence and potential role within oral microbial communities (Kolenbrander, 2000).

The medicinal properties of *Ocimum sanctum* have been studied in invitro, animal, and human experiments. These studies reveal that Tulsi has a unique property that include: Antimicrobial, anti-oxidant, anti-cataract, anti-inflammatory and anti-coagulant activities. The general mechanism of action of eugenol on bacterial biofilm includes inhibition of biofilm formation and reduced viability of biofilm-forming cells (Taylor *et al.,* 2005).

Camellia sinensis has a strong antioxidant power through its polyphenolic chemical constituents, beneficial in several clinical conditions such as dental caries, gingivitis, periodontitis, and halitosis, in addition to neuroprotection in the oral cavity. Catechins make up the majority of flavonoids in green tea, accounting for 80-90% of its composition and approximately 40% of its water-soluble solids. The four main catechins found in green tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG). The most abundant catechin is EGCG (~60%), and the next most abundant is EGC (~20%), then ECG (~14%), and EC (~6%) (Chacko *et al.,* 2010).

Neem leaves have been reported to exhibit antibacterial, antifungal, hepatoprotective, anti-ulcer, anti-fertility and anti-nociceptive activity. The phytochemical constituents present in neem are Nimbidin, Nimbin, Nimbolide, Azadirachtin, gallic acid, epicatechin, catechin, and margolone (Wylie & Merrell, 2022). All these exhibits potent antibacterial activity. Nimbidin exhibits potent antibacterial activity against a wide range of oral pathogens implicated in dental plaque formation.

II. RESEARCH METHODOLOGY

SAMPLE COLLECTION, ISOLATION & CHARACTERIZATION

Microorganisms that cause dental plaque (S1-S10) were isolated from patients with dental caries who were treated at Smile Dental Hospital and Implant Care in Hyderabad. Additionally, standard strain, *Candida albicans* (MTCC 227), recognized as a recurrent oral biofilm-forming yeast, was procured from IMTECH, Chandigarh. The isolates were inoculated onto Czapekdox agar for fungal cultures and *Alternaria* was isolated in the study. *Candida albicans* (MTCC227) was initially inoculated in Yeast extract Potato Dextrose (YPD) Broth, streaked on Malt Yeast agar, and incubated at 37ºC for 24 h.

PREPARATION OF METHANOLIC EXTRACTS OF *OCIMUM SANCTUM, CAMELLIA SINENSIS* AND *AZADIRACHTA INDICA*

Preparation of methanolic extract using Soxhlet extraction:

The powdered leaves of *Ocimum sanctum, Camellia sinensis*, and *Azadirachta indica* (22g, 50g, and 26g respectively) were each extracted with 300ml methanol in a Soxhlet extractor until colorless. Methanolic extracts were concentrated using a rotary vacuum evaporator and stored in screw cap tubes in the refrigerator (Hashim *et al.,* 2021).

PHYTOCHEMICAL ANALYSIS OF *OCIMUM SANCTUM, CAMELLIA SINENSIS* AND *AZADIRACHTA INDICA*

The methanolic extracts of *Ocimum sanctum, Camellia sinensis & Azadirachta indica* leaves were subjected to quantitative analysis for various phytochemicals, including alkaloids, tannins, proteins, phytosterols, phenols, saponins, glycosides, acids, quinones, terpenoids, reducing sugar, flavonoids, and carbohydrates (Benisheikh *et al*. 2019; Geoffrey *et al.* n.d.; Latteef n.d.; Sayuri Nagano and Batalini 2021; Seriana *et al.* 2021). **(Table 1)**

Table 1: Phytochemical screening of methanolic extracts of Ocimum sanctum, *Camellia sinensis* and *Azadirachta indica*

Test	Observation
Alkaloids	Wagner's reagent was added to methanolic extracts, and the appearance of a brown to flocculent precipitate indicated the presence of alkaloids.
Tannins/Phenols	FeCl3 solution was added to the extracts, and the formation of a greenish-black colour confirmed the presence of tannins or phenols.
Proteins	Methanolic extracts were mixed with concentrated HNO3, and the observation of a white or yellow precipitate indicated the presence of proteins.
Phytosterols	Salkowski reaction was performed, and the appearance of red or brown colouration indicated the presence of phytosterols
Saponins	The formation of froth upon mixing extracts with sodium bicarbonate and distilled water indicated the presence of saponins
Glycosides	Treatment with glacial acetic acid and FeCl3 solution led the emergence of a reddish-brown precipitate, confirming the presence of glycosides.
Acids	Effervescence upon the addition of sodium bicarbonate solution confirmed the presence of acids.
Quinones	The appearance of a red colour upon addition of concentrated H2SO4 indicated the presence of quinones
Terpenoids	The manifestation of reddish-brown colouration upon treatment with chloroform and concentrated H_2SO_4 confirmed the presence of terpenoids.
Reducing Sugar	Heating extracts with Benedict's reagent resulted in a brown-to-red colouration, indicating the presence of reducing sugar.
Flavonoids	The addition of concentrated H_2SO_4 led to a yellowish-orange colouration, confirming the presence of flavonoids.
Carbohydrates	Upon heating with Fehling's reagent, the appearance of a reddish-orange precipitate confirmed the presence of carbohydrates

PURIFICATION OF THE ACTIVE COMPONENT OF *OCIMUM SANCTUM, CAMELLIA SINENSIS* AND *AZADIRACHTA INDICA* USING THIN LAYER CHROMATOGRAPHY:

Purification of Eugenol from Tulsi leaf extract using Chromatographic Methods:

To extract the Eugenol from Tulsi extract, thin-layer chromatography was utilized. TLC plates (20 x 20 cm) with flexible plastic backing and silica gel were obtained. The solvent used is toluene & ethyl acetate in the ratio of 9.3: 0.7. Examination under UV light at 254 nm revealed an orange red spot, indicating the presence of eugenol. (James V. De Francesco, 2021).

Purification of Catechin from Green tea extract using Chromatographic Methods:

Thin-layer chromatography (TLC) was used to identify Catechin in green tea extract. A solution of acetic acid and chloroform (1:9 ratio) was used for separation. Examination under UV light at 280nm revealed a dark orangish-pink spot, indicating the presence of Catechin. This TLC-UV method ensures precise identification of Catechin in green tea extract (Alasadiy, 2013).

Purification of Nimbidin from Neem leaf extract using Chromatographic Methods:

Thin-layer chromatography (TLC) was used to extract Nimbidin from Neem extract. The development tank contained an 8:2 mixture of ethanol and water. Nimbidin was visualized under UV light at 560nm, appearing as yellow or orange fluorescent spots (K. Gilbert Ross Rex *et al.,* 2021).

BIOFILM PRODUCTION:

Congo red agar method:

Brain Heart Infusion agar and Congo red stain were autoclaved, poured into Petri plates, and allowed to solidify. The isolates and *Candida albicans* (MTCC227) were streaked on Congo red agar plates and incubated inverted at 37ºC for 24-48h. Black colonies with a dry crystalline consistency indicated biofilm production. (Sahra Kırmusaoğlu *et al*, 2018).

Tube detection method:

10 ml aliquot of Trypticase soy broth with 1% glucose was inoculated with the test organisms. After 24 h incubation at 37ºC, cultures were decanted and washed with phosphate buffer saline. Tubes were dried, stained with 0.1% crystal violet, and excess stain was washed away. Positive biofilm formation was assessed based on intensity: absent, weak, moderate, or strong (Kala Harika *et al.,* 2020).

Microtiter plate assay:

10 ml of Trypticase soy broth with 1% glucose was inoculated with test organisms separately. After 24 h incubation at 37ºC, cultures were diluted 1:100 with fresh medium. Diluted cultures were transferred to 96-well plates and incubated for another 24 h. Wells were washed with phosphate buffer saline to remove free-floating bacteria. Adherent biofilms were fixed, stained with crystal violet, and OD was measured at 630nm using a micro-ELISA auto reader. (Kala Harika *et al.,* 2020).

ANTIMICROBIAL ACTIVITY OF THE EXTRACTS AGAINST ORAL BIOFILM-FORMING MICROORGANISMS BY AGAR WELL DIFFUSION METHOD:

Mueller-Hinton agar plates were prepared, supplemented with Streptomycin to prevent bacterial growth. The fungal suspension was spread on the agar. Antifungal solutions of varying concentrations were added to wells in the agar. After incubation at RT for 3-5 days, visible zones of inhibition around wells indicate susceptibility. The diameter of these zones was measured. (D.Al-asadiy, 2013).

STATISTICAL ANALYSIS

The data was subjected for statistical analysis using SPSS and the Tukey's HSD procedure was used for pairwise comparisons within ANOVA data. The level of significance used is α=0.05. (Smolarek PC *et al.,* 2015)

HPLC ANALYSIS

The present study employed high-performance liquid chromatography (HPLC) for the analysis of standards and samples. The Hypersil Gold (150mm) column was used for the analysis. The mobile phase consisted of acetonitrile and water in a ratio of 90:10, supplemented with a buffer of 0.1% Formic acid in water. The flow rate was set at 1 ml/min, while the absorption wavelength was fixed at 225 nm. The sample injection volume was 1.00 microlitre, and the entire method was run for 24 min. Peak identification was done by comparing their retention times. (Theerapong Theppakorn & Sirirung Wongsakul, 2012).

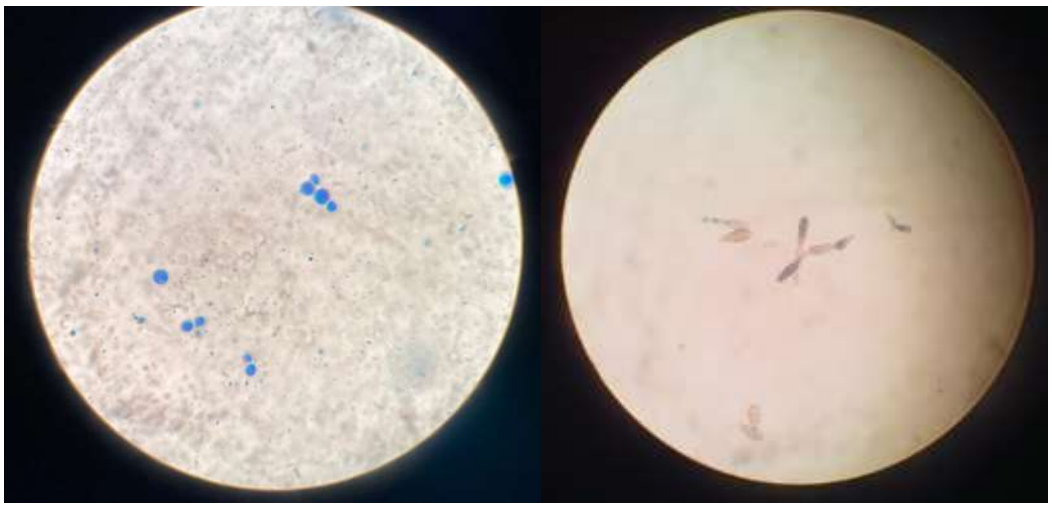
GREEN TEA MOUTHWASH

Two grams of powdered *Camellia sinensis* were diluted in 100 mL of distilled water and boiled for 10 min at 75^0C. The resulting solution was filtered and concentrated. To enhance properties, 0.3 g of sucrose and 0.01 g of sodium lauryl sulfate were added as a foaming agent. Sodium metabisulfite, a preservative, was mixed into 10 mL of the extract. Homogenization was performed on a magnetic stirrer for uniformity.

III. RESULTS AND DISCUSSION

ISOLATION & CULTURAL CHARACTERISTICS:

Cultural characteristics of the fungal samples such as the arrangement of colony, size, shape and margin were studied on Czapekdox Agar. Upon staining by Lactophenol cotton blue, the cells of *Candida albicans* appeared round to oval, ranging in size from 2 to 4 um. Based on the morphological characteristics and staining with Lactophenol cotton blue the fungal isolate was identified as *Alternaria.* The fungi *Alternaria* was characterized by its septate and branched mycelium. Its conidia exhibited a pyriform shape, with a long beak and possess transverse and longitudinal septa as shown in the Fig 1



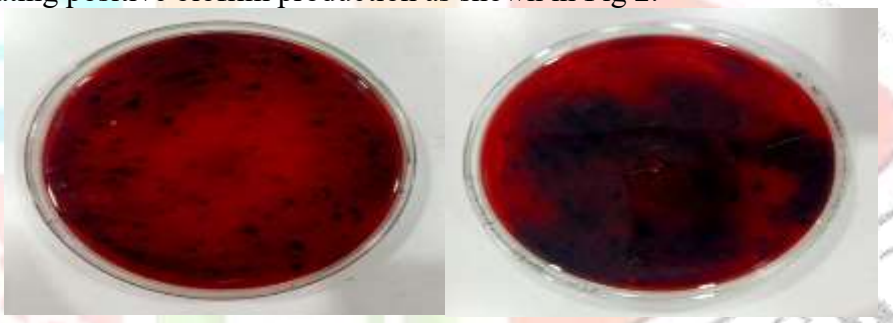
(A) **(B)**

Fig 1: Microscopic Field view of *(A) Candida albicans and (B) Alternaria* by Lactophenol Cotton Blue Staining Method

BIOFILM PRODUCTION:

Congo Red Agar Method

The Congo Red Agar (CRA) method detects biofilm-producing microorganisms based on colony colour changes after 24 h incubation at 37°C. Biofilm-producing colonies appear black and dry, while non-biofilm producers are pink. *Candida albicans* and *Alternaria* showed moderate biofilm production, observed as rough, black colonies, indicating positive biofilm production as shown in Fig 2.

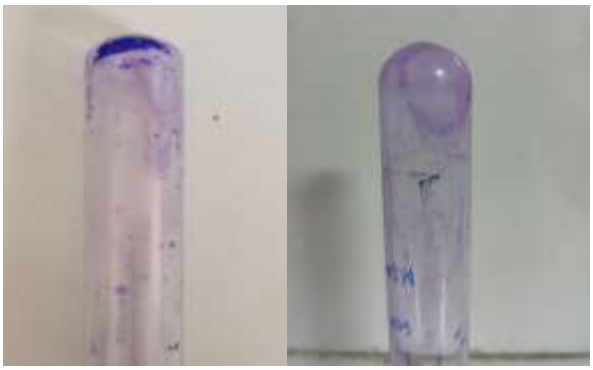


(A) **(B)**

Fig 2: Analysis of Biofilm production in (A) *Candida albicans* and (B) *Alternaria* using Congo Red Agar Method

Tube Detection Method

Biofilm formation was assessed visually, graded as absent, moderate, or strong. *Candida* displayed high levels, resulting in a dark blue stain on tube walls while *Alternaria* showed minimal biofilm production. **(Fig 3)**.



(A) **(B)**

Fig 3: Biofilm production by Tube Detection Method in (A) *Candida albicans* and (B) *Alternaria*

Microtiter Plate Assay

The microtiter plate was subjected to optical density measurements at a wavelength of 630 nm and subsequently examined. The recorded optical density values for the individual wells which indicate the formation of biofilm are shown in **Table 2**.

Table 2: Microtiter Plate Assay

FUNGAL SAMPLE	MICROTITRE PLATE METHOD OD AT 630 nm
Blank	**0.057**
Candida albicans	**0.078- 0.057= 0.021**
Alternaria	**0.091- 0.057= 0.034**

QUALITATIVE ANALYSIS

PHYTOCHEMICAL ANALYSIS OF *OCIMUM SANCTUM, CAMELLIA SINENSIS* AND *AZADIRACHTA INDICA*

Tulsi extract showed the presence of alkaloids, phenols, saponins, glycosides, tannins, Terpenoids whereas proteins, phytosterols, quinones, reducing sugars and carbohydrates were absent in the extract. Green tea extracts displayed positive results for alkaloids, phytosterols, phenols, saponins, glycosides, tannins, quinones, terpenoids, reducing sugars while proteins, and carbohydrates were absent. Neem extracts exhibited positive results for alkaloids, phytosterols, phenols, saponins, acids, glycosides, tannins, quinones, and terpenoids, and showed negative results for proteins, reducing sugars, and carbohydrates as shown in **Table 3.**

Table 3: Phytochemical Analysis of *Ocimum sanctum, Camellia sinensis* And *Azadirachta indica*

PHYTOCHEMICALS	OCIMUM SANCTUM	CAMELLIA SINENSIS	AZADIRACHTA INDICA
ALKALOIDS	Positive	Positive	Positive
PROTEINS	Negative	Negative	Negative
PHYTOSTEROLS	Negative	Positive	Positive
PHENOLS	Positive	Positive	Positive
SAPONINS	Positive	Positive	Positive
GLYCOSIDES	Positive	Positive	Positive
TANNINS	Positive	Positive	Positive
QUINONES	Negative	Positive	Positive
TERPENOIDS	Positive	Positive	Positive
REDUCING SUGAR	Negative	Positive	Negative
CARBOHYDRATES	Negative	Negative	Negative

THIN LAYER CHROMATOGRAPHY

Eugenol was identified by performing TLC of Tulsi extract. Orange red spots were visualized under 254 nm UV light. A comparison of the Rf values of the spots indicates a positive identification of eugenol as component in the Tulsi leaves extract. As per the Literature by Soran, *et al.,* 2016 the retention time for Eugenol is 5-6. Therefore, this proves the presence of Eugenol.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Rf= 2.3/ 4= 5.75

Rf= 5.75

Catechin was identified through the observation of an orange pink fluorescent spot under UV light at 280nm (Alasadiy 2013). Green tea, along with its constituent catechins, is widely recognized for its antioxidant properties, with implications for diverse conditions associated with reactive oxygen species (ROS).

The confirmation of Nimbidin was achieved through the visualisation of an orange florescent spot under UV light at 560nm (K Gilber Ross *et al.,* 2021). Nimbin, identified as a triterpenoid compound derived from Neem. Scientific literature, notably the work of W. Krauss *et al*. in 1995, attests to the properties attributed to nimbin. These encompass anti-inflammatory, antipyretic, fungicidal, antihistamine, and antiseptic attributes, contributing to the compound's pharmacological significance within the context of neem-derived products as shown in Fig 4.

(A) (B) (C)

Fig 4: Thin Layer Chromatography (TLC) Assessment of (A) Eugenol in *Ocimum sanctum* (B) Catechin in *Camellia sinensis* (C) Nimbidin in *Azadirachta indica*

ANTIMICROBIAL SENSITIVITY TESTING

The extracts of *Camellia sinensis, Ocimum sanctum*, *Azadirachta indica* and methanol as solvent were used to check their inhibitory effects against *Candida and Alternaria*. An antifungal agent, Flucanozole was used in the current study to compare its activity against the extracts. According to the study, *Camellia sinensis* demonstrated a pronounced zone diameter, succeeded by *Ocimum sanctum and Azadirachta indica*. These outcomes align with the study conducted by Gok B *et al.,* in 2020, wherein *Camellia sinensis* exhibited noteworthy inhibition against oral bacterial biofilms. It was observed that Flucanozole had no antimicrobial activity against *Candida albicans* whereas Flucanozole exhibited significant antifungal effect against *Alternaria* as observed in **Table 4.1 & Table 4.2 and Fig 5**.

Table 4.1: Comparative analysis of antimicrobial activity of Leaf extracts and Flucanozole against *Candida albicans*

PLANT EXTRACTS	***OCIMUM SANCTUM***	***CAMELLIA SINENSIS***	***AZADIRACHTA INDICA***	**METHANOL (SOLVENT)**	**FLUCANOZOLE**
Conc.	**Zone Diameter in mm**				
0.5 mg/ml	19	27	12	0	0
1.0 mg/ml	11	30	13	0	0
1.5 mg/ml	16	43	14	0	0
2.0 mg/ml	13	47	15	0	0

Table 4.2: Comparative analysis of antimicrobial activity of Leaf extracts and Flucanozole against *Alternaria*

PLANT EXTRACTS	***OCIMUM SANCTUM***	***CAMELLIA SINENSIS***	***AZADIRACHTA INDICA***	**METHANOL (SOLVENT)**	**FLUCANOZOLE**
Conc.	**Zone Diameter in mm**				
0.5 mg/ml	0	16	0	0	25
1.0 mg/ml	0	17	0	0	22
1.5 mg/ml	0	30	25	0	22
2.0 mg/ml	0	36	0	0	24

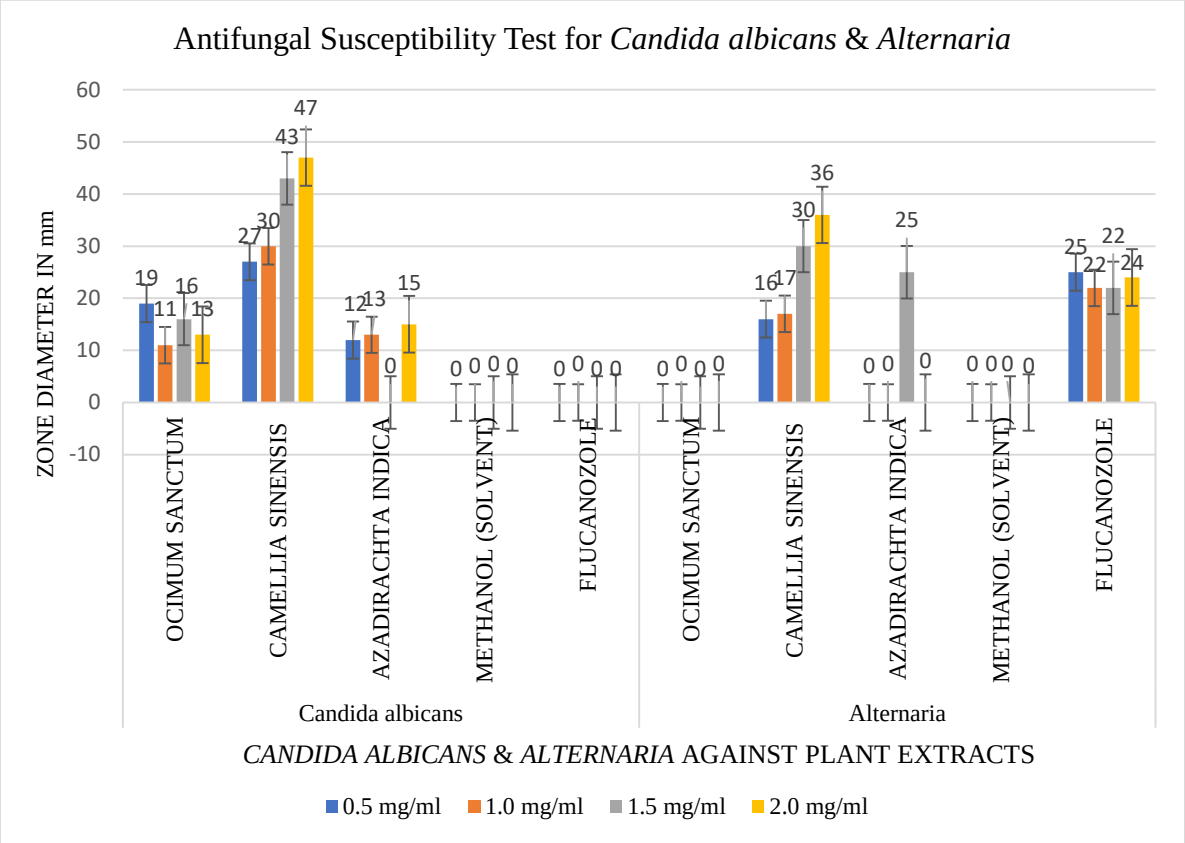


Fig 5: Graphical representation of Antifungal Susceptibility Test

DESCRIPTIVE STATISTICAL ANALYSIS

Table 5: Mean, Standard error & Standard Deviation of Zone of Inhibition by Green Tea extract against both *Candida albicans* & *Alternaria*

Candida albicans		***Alternaria***	
MEAN	3.675	**MEAN**	2.475
STANDARD ERROR	0.487126	**STANDARD ERROR**	0.492231
STANDARD DEVIATION	0.974252	**STANDARD DEVIATION**	0.984463

The multiple comparison tests between treatments versus control groups were evaluated by one-way analysis of variance (ANOVA), and the Tukey with a significance level at 5%.

ANOVA indicated that *Alternaria* responded differently to *Ocimum sanctum*, *Camellia sinensis, Azadirachta indica* and Flucanazole ($p=.000024$; $p < 0.05$) at a concentration of 1.5mg/mL. A *post-hoc* Tukey's test showed that the difference between the antimicrobial activity of the extracts *Ocimum sanctum, Camellia sinensis, Azadirachta indica* was significant with p value of 0.00007 ($p<0.05$). *Camellia sinensis* was more effective than Flucanazole against *Alternaria* ($p = .00006$) and the difference between antimicrobial activity of *Azadirachta indica* and Flucanazole against *Alternaria* was not significant ($p = .99156$). Similar results were observed against *Candida albicans* with $p < 0.00001$. The study shows that *Camellia sinensis* has good antimicrobial activity against *Alternaria sps* and *Candida albicans.*

HPLC ANALYSIS OF GREEN TEA

The isolation and quantitative analysis of phytochemicals within green tea extract were conducted utilizing High-Performance Liquid Chromatography (HPLC) coupled with Photodiode Array (PDA) detection. The chromatogram depicting the results is illustrated in Fig 6. The quantification of the sample was achieved by comparing the retention time and peak areas with those obtained from a standard reference paper (Theerapong Theppakorn *et al.,* 2012). The retention time of the peaks were compared to a standard paper. Catechin (RT- 9.710), Epicatechin (RT- 10.351) and Epigallocatechin-3-gallate (RT- 12.435) were identified as shown in Table 6.

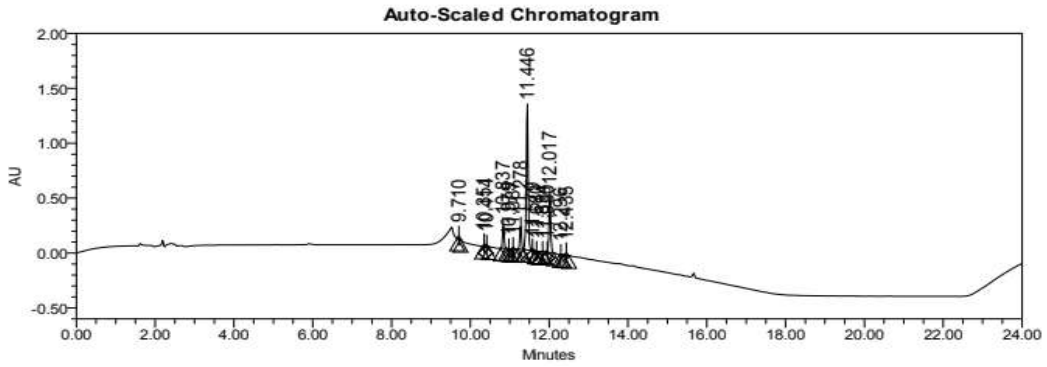


Figure 6: HPLC Chromatogram of *Camellia sinensis*

Table 6: Major Catechins from *Camellia sinensis* and their Retention Time evaluated via HPLC

S.NO	NAME	RETENTION TIME	AREA	% AREA
1.	Catechin (C)	9.710	84131	1.12
2.	Epicatechin (EC)	10.351	72617	0.96
3.	Epigallocatechin-3-gallate (EGCG)	12.435	93538	1.24

SYNTHESIS OF *CAMELLIA SINENSIS* (GREEN TEA) MOUTHWASH

A mouthwash was synthesized using green tea extract based on its effective antimicrobial activity. The formulation included other components and resulted in a dark green tea mouthwash with visible froth.

COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY OF GREEN TEA (SYNTHESIZED) & COMMERCIAL MOUTHWASH:

The antimicrobial activity of the synthesized green tea mouthwash was compared to a commercial mouthwash (Chlorhexidine) at different concentrations**.** Wells were bored, and different concentrations of the synthesized and commercial mouthwash (25, 50, and 100 µL) were introduced and checked against *Candida* and *Alternaria.*

(A) **(B)**

Fig 7: Comparative analysis of Green Tea Mouthwash against Commercial mouthwash i.e., Chlorhexidine against (A) *Candida albicans* & (B) *Alternaria*

Table 7: Comparative Analysis of Antimicrobial activity of Commercial Mouthwash and Green Tea Mouthwash at Different Concentrations against *Candida albicans* & *Alternaria*

S.NO	CONCS	*CANDIDA ALBICANS*		*ALTERNARIA*	
		ZONE DIAMETER IN CMS			
		CHLORHEXIDINE	GREEN TEA MOUTHWASH	CHLORHEXIDINE	GREEN TEA MOUTHWASH
1.	25µl	1.9 cm	2.0 cm	2.6 cm	0 cm
2.	50 µl	2.0 cm	2.7 cm	4.1 cm	2.1 cm
3.	100 µl	2.6 cm	3.2 cm	3.6 cm	3.5 cm

As observed in the **Table 7 & Fig 7**, the findings indicate that Green tea mouthwash is comparatively more effective in inhibiting *Candida albicans* in comparison to Chlorhexidine, with increasing concentrations. Additionally, it was observed that *Alternaria* also was inhibited by Green tea mouthwash, albeit not as significant as that observed in the case of Chlorhexidine.

IV. CONCLUSION

Biofilms, prevalent in moist environments, are complex microbial communities with significant implications for health, notably in dental plaques. In India, the tradition of herbal remedies, including Tulsi, Green Tea, and Neem, is deeply ingrained. These botanicals have demonstrated efficacy in treating various ailments. Green Tea, Neem, and Tulsi are strategically integrated to address oral biofilms due to their accessibility and effectiveness. Green Tea, in particular, shows strong inhibition against fungal biofilms due to its principle phytochemical, catechin. This study isolated *Candida albicans* and *Alternaria* from dental samples and identified key phytochemicals in Tulsi, Green Tea, and Neem. Thin Layer Chromatography was used to purify Eugenol, Catechins, and Nimbidin from these plants. Antifungal sensitivity tests revealed Green Tea's superior efficacy in inhibiting *Candida albicans* and *Alternaria* compared to Tulsi and Neem. The findings suggest Green Tea as a potential treatment for fungal oral biofilms, indicating the promise of natural compounds in microbial infection treatment. Future research could explore specialized formulations like Green Tea-based mouthwashes to bridge traditional knowledge with modern oral healthcare needs.

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