PROFORMA FOR REGISTRATION OF DISSERTATION

(IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF DENTAL SURGERY)

HEMWATI NANDAN BAHUGUNA UTTARAKHAND MEDICAL EDUCATION UNIVERSITY, DEHRADUN

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

SEEMA DENTAL COLLEGE AND HOSPITAL, RISHIKESH

"COMPARATIVE EVALUATION OF EFFECTIVENESS OF DIFFERENT IRRIGATING SOLUTIONS ON SMEAR LAYER REMOVAL UNDER SCANNING ELECTRON MICROSCOPE (SEM): AN EX VIVO STUDY.

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SUBMITTED BY:

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8 MATERIALS AND METHOD:

8.1 SOURCE OF DATA: 100 Freshly Extracted Human Permanent Teeth will be collected from the Department of Oral & Maxillofacial Surgery, Seema Dental College & Hospital. Institutional Consent Form Protocol for extracted human permanent teeth for research study purpose will be followed. The herbal irrigating solutions will be collected from the Department of Biomedical Sciences, Kumaon University, Nainital, Uttarakhand.

8.2 METHOD OF COLLECTION OF DATA:

(Including Sampling Procedure if Any)

INCLUSION CRITERIA:

1. Caries Free teeth.
2. Teeth with intact and mature root apices.
3. Extracted teeth that are periodontally compromised.

EXCLUSION CRITERIA:

1. Carious Teeth.
2. Tooth Cracks/ Fracture/ Restored Teeth.
3. Primary Teeth.
4. Teeth with Any Developmental Anomalies.

8.3 PREPARATION OF SAMPLES:

100 freshly extracted human permanent teeth will be collected from Department of Oral and Maxillofacial Surgery, Seema Dental College and Hospital to be used in this study. The herbal irrigating solutions will be collected from the Department of Biomedical Sciences, Kumaon University, Nainital, Uttarakhand. The CDC guidelines for infection control in dental health care setting 2003 will be followed for the preparation of samples. These teeth will be scrubbed with detergent using ultrasonic cleaner to clean off visible blood and gross debris, they will be maintained in a well hydrated state containing 10% formalin. The teeth will then be disinfected and sterilized by first immersion in 10% Formalin for 7 days followed by autoclaving at 121°C, 15 psi for 30 minutes to allow safe handling. OSHA guidelines 2014 for disposal of extracted teeth will be followed. The samples will be disposed off in medical waste container that uses an incinerator for final disposal. Only the teeth with intact and mature root apices will be included in the study. Standardized radiographs will be taken in a bucco-lingual and mesio-distal dimension before the instrumentation. The specimens will be placed in a radiographic mount. Coronal access will be achieved by using Endo-Access & Endo-Z burs to obtain a straight-line access followed by Working length determination and Biomechanical

preparation.

SAMPLE SIZE ESTIMATION:

Sample size estimation was done by using **GPower software (version 3.0).** Sample size was estimated for **One way ANOVA test.**

A minimum total sample size of 100 was found to be sufficient for an alpha of 0.05, power of 80%, 0.45 as effect size (as obtained for mean amount of debris present among different study groups from the similar article). Sample size was further divided as 20 into five groups.

Reference: Armitage, P., Berry, G., & Matthews, J. (2002). Statistical methods in medical Research.

F tests: ANOVA: Fixed effects, omnibus, one-way.

Analysis: A priori: Compute required sample size.

Input:	Effect size f	= 0.36
	α err prob	= 0.05
	Power (1-β err prob)	= 0.80
	Number of groups	= 5
Output:	Noncentrality parameter λ	= 12.9600000
	Critical F	= 2.4674936
	Numerator df	= 4
	Denominator df	= 95
	Total sample size	= 100
	Actual power	= 0.8144325

The five experimental groups on the basis of irrigation solution used after canal preparation will be, namely:

Group A: 17% Ethylenediaminetetraacetic acid (EDTA)

Group B: Neem Extract

Group C: Tulsi Extract

Group D: Green Tea Extract

Group E: Turmeric Extract

All specimens in a group will be treated by a single operator and sent for Scanning Electron Microscopic study. Photomicrographs of dentinal walls will be produced. The amount of debris and dentinal tubule diameter will be evaluated and values will be subjected to statistical analysis.

8.4 STATISTICAL ANALYSIS:

Data will be analysed using Statistical Package for Social Sciences (SPSS) version 21. The study variables which will be categorical, will be summarized as proportions and frequencies. Graphs will be prepared on Microsoft Excel. For intergroup comparison of mean surface roughness one way ANOVA / Kruskal Wallis test will be used depending on the normality of the data. Similarly post hoc pairwise comparison will be done using Post Hoc Tukey's test or Mann Whitney U test. The level of statistical significance will be set at 0.05.

8.5 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

This study is Ex-Vivo so it does not require any investigation.

8.6 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN THE CASE OF 8.5?

YES

PROFORMA FOR SHORT STUDY

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

SEEMA DENTAL COLLEGE AND HOSPITAL, RISHIKESH

"TO CHECK THE EFFICACY OF NICOTINE PASTILLE (NOBACCO) FOR HABIT CESSATION IN TOBACCO USERS OF UTTARAKHAND POPULATION"

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1.	**NAME OF THE CANDIDATE & ADDRESS** **(IN BLOCK LETTERS)**	**DR. SUSHMA DARNAL** 2nd YEAR POST GRADUATE STUDENT DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY SEEMA DENTAL COLLEGE AND HOSPITAL, VIRBHADRA ROAD, RISHIKESH-249203, UTTARAKHAND.
2.	**COURSE OF STUDY & SUBJECT**	MDS 2nd YEAR SUBJECT-ORAL MEDICINE AND RADIOLOGY.
3.	**DATE OF ADMISSION TO COURSE**	1ST NOVEMBER 2022-2025
4.	**TITLE**	TO CHECK THE EFFICACY OF NICOTINE PASTILLE (NOBACCO) FOR HABIT CESSATION IN TOBACCO USERS OF UTTARAKHAND POPULATION.

5	**BRIEF RESUME OF THE INTENDED WORK:**

5.1 Need for the study:

Today tobacco use is the single greatest preventable cause of death in the world. Tobacco use is often incorrectly perceived to be solely a personal choice. This is contradicted by the fact that when fully aware of the health impact, most tobacco users want to quit but find it difficult to stop due to the addictiveness of nicotine.[1]

Despite public health efforts to decrease tobacco use, 21% of the adult population still smokes. Of these, 70% would like to quit, and 42.5% of smokers make a quit attempt each year. The quit rate for those utilizing no form of treatment is approximately 5% per year, making the need to increase smoking cessation rates a top public health priority.[2]

Nicotine replacement therapy (NRT) is the most widely used pharmacological therapy for smoking cessation. The efficacy of NRT has been proven in many clinical trials, approximately doubling the success rates for quitting compared withplacebo.5 Nevertheless, most smoking cessation efforts are attempted without benefit of treatment. Quid is defined as a substance or mixture of substances placed in the mouth or chewed, remaining in contact with the mucosa, and usually containing one or both of the two basic ingredients, tobacco and/or areca nut in raw or processed forms.[3]

A number of nicotine replacement products are available: gum, patch, nasal spray, and inhaler. Oral transmucosal nicotine (OT-NIC) is a novel nicotine delivery system consisting of a lozenge, containing 4 mg of nicotine, mounted on a plastic handle. The OT-NIC unit is placed in the cheek pouch and the lozenge allowed to dissolve. As the lozenge dissolves, it rapidly releases nicotine, which is absorbed through the buccal mucosa into the systemic circulation.[4]

So, the use of OT-NIC would result in significant suppression of withdrawal symptoms, would be well tolerated with no major safety differences for this delivery system compared to nicotine patch or gum.[4]

The objective of the study is to check the efficacy of nicotine pastille (NOBACCO) for habit cessation in tobacco users of Uttarakhand population.

5.2 Aim:

The aim of this study is to evaluate the efficacy of nicotine pastille (NOBACCO) for habit cessation in tobacco users of Uttarakhand population.

5.3: Objectives of the study:

1. To identify the patients with habit of tobacco use.

2. To determine the frequency of tobacco consumption.

3. To check the efficacy of nicotine pastille (NOBACCO) for habit cessation.

5.4: Review of Literature:

Brian G. Danaher et al (2014)[5]

A study on total of 407 smokeless tobacco users who wanted to quit. They were screened online, and randomly assigned to one of two conditions: (a) the interactive MyLastDip Web-based intervention (Web Only; n =202), or (b) the website plus the offer of nicotine lozenges (Web + Lozenge; n = 205). The study concluded that consistent with previous research, the MyLastDip Web-based tobacco cessation intervention encouraged long-term levels of tobacco and smokeless tobacco abstinence. The addition of nicotine lozenges significantly improved both participant engagement and self-reported 7-day point prevalence tobacco abstinence at 3 months and when considering 3- and 6-month repeated point prevalence tobacco abstinence.

Herbert H. Severson et al (2015)[6]

A randomized trial was conducted on 1067 smokeless tobacco users online and they were randomly assigned in 1 of 3 conditions:(a) a lozenge group (n = 356), who were mailed 4-mg nicotine lozenges; (b) a coach calls group (n = 354), who were offered 3 coaching phone calls; or (c) a lozenge + coach calls group (N = 357), who received both lozenges and coaching calls. Additionally, all participants were mailed self-help materials. Self-reported tobacco abstinence was assessed at 3 and 6 months after randomization. The result concluded that ccombining nicotine lozenges and phone counselling significantly increased tobacco abstinence rates compared with either intervention alone, whereas coach calls and

lozenges were equivalent. The study confirms the high tobacco abstinence rates for self-help ST cessation interventions and offers guidance to providing tobacco treatment to ST users.

6 MATERIALS AND METHODS:

6.1 Source of data:

The patients to be studied will be selected from the outpatient Department of Oral Medicine & Radiology, Seema Dental College and Hospital, Rishikesh, Uttarakhand.

Study Design: Interventional clinical Study

Study Period: April 2024 to August 2024

Place of study: Seema Dental College and Hospital, Rishikesh

Sampling design, method and size:

Sample size was determined based on the expected prevalence of tobacco abstinence using the following formula:

$$N = \frac{Z\alpha/22\ Pq}{d2}$$

where,

N= Sample Size

Zα/2 = 1.44 at 85% Confidence Interval [15% type I error].

P = 25% [Ebbert JO et al, Nicotine & Tobacco Research, 2009]

q = 1 - p

d = allowable error of 15%

So,

$$N = \frac{1.442 \times 0.25 \times 0.75}{(0.15)2}$$

N= 17.25 [non-response rate of 20%]

N – 21.5 ~ 22

A minimum sample size of 22 participants will be required for the present study.

Inclusion criteria:

1. Patients who were willing to participate in the study.
2. Patients with a history of smoking tobacco.
3. Patients with a history of chewing tobacco.

Exclusion criteria:

1. Patients who were not willing to participate in the study.
2. Discontinuation of smoking habit over a period of 5 years or more.
3. Discontinuation of follow-up.

Informed consent: Required

6.2 Method of collection of data

The patients to be studied will be selected from the outpatient Department of Oral Medicine & Radiology, Seema Dental College and Hospital, Rishikesh, Uttarakhand.

Materials

1. A minimum sample size of 22 participants will be required for the present study.
2. NOBACCO pastille.

Methodology

The patients will be provided with a form of basic demographic details and habit history.

Statistical analysis:

Data will be entered into the Excel sheet. Data will be analyzed using SPSS (Statistical Package for Social Sciences) 22.0 version, IBM Corp. Descriptive statistics will be performed. Categorical data will be compared using the Chi-Square test and McNemar test. The level of significance will be set at $P \leq 0.05$.

	6.3 Does the study require any investigation or interventions to be conducted on patients or other humans or animals? Yes **6.4 Has ethical clearance been obtained from your institution in case of 6.3?** Yes

7	**List of references:**

1. Danaher BG, Severson HH, Crowley R, van Meter N, Tyler MS, Widdop C, Lichtenstein E, Ebbert JO. Randomized controlled trial examining the adjunctive use of nicotine lozenges with MyLastDip: an eHealth smokeless tobacco cessation intervention. Internet Interventions. 2015 Mar 1;2(1):69-76.
2. Pack QR, Jorenby DE, Fiore MC, Jackson T, Weston P, Piper ME, Baker TB. A comparison of the nicotine lozenge and nicotine gum an effectiveness randomized controlled trial. WMJ: official publication of the State Medical Society of Wisconsin. 2008 Aug;107(5):237.
3. Shiffman S, Dresler CM, Hajek P, Gilburt SJ, Targett DA, Strahs KR. Efficacy of a nicotine lozenge for smoking cessation. Archives of Internal Medicine. 2002 Jun 10;162(11):1267-76.
4. Muramoto ML, Ranger-Moore J, Leischow SJ. Efficacy of oral transmucosal nicotine lozenge for suppression of withdrawal symptoms in smoking abstinence. Nicotine & Tobacco Research. 2003 Jan 1;5(2):223-30.
5. Severson HH, Danaher BG, Ebbert JO, Van Meter N, Lichtenstein E, Widdop C, Crowley R, Akers L, Seeley JR. Randomized trial of nicotine lozenges and phone counseling for smokeless tobacco cessation. Nicotine & Tobacco Research. 2015 Mar 1;17(3):309-15.
6. Wadgave U, Nagesh L. Nicotine replacement therapy: an overview. International journal of health sciences. 2016 Jul;10(3):425.

ANNEXURE-1

SEEMA DENTAL COLLEGE AND HOSPITAL

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

CONSENT FORM

I ……. Resident of ……. District Uttarakhand give consent for my inclusion in the study "To check the efficacy of nicotine pastille (NOBACCO) for habit cessation in tobacco users of Uttarakhand population" conducted by PG student Dr. Sushma Darnal under the guidance of Dr. S Karpagavalli, Professor and Head and Co Guide Dr. Avnish Singh, Professor and Head, in the Department of Public Health and Dentistry, Seema Dental College and Hospital, Rishikesh.

Name Signature with Date

सीमा डेंटल कॉलेज और अस्पताल

मौखिक चिकित्सा और रेडियोलॉजी विभाग

सहमति पत्र

मैं ..... का निवासी हूँ जिला उत्तराखंड, उत्तराखंड जनसंख्या के तंबाकू उपयोगकर्ताओं में अभ्यस्ति निवृत्ति के लिए निकोटीन पास्टिल (नोबैको) के प्रभाव की जाँच करने के लिए "To check the efficacy of nicotine pastille (NOBACCO) for habit cessation in tobacco users of Uttarakhand population" अध्ययन में शामिल होने के लिए स्वीकृति देता हूँ, जो सीमा डेंटल कॉलेज और अस्पताल के लोक स्वास्थ्य और दन्त विभाग में डॉ। सुषमा डर्नाल के मार्गदर्शन में पीजी छात्र डॉ। एस कार्पगवल्ली, प्रोफेसर और हेड और सह सलाहकार डॉ। अवनीश सिंह, प्रोफेसर और हेड द्वारा आयोजित किया गया है।

नाम: हस्ताक्षर तथा तारीख।:

ANNEXURE-2

SOCIO DEMOGRAPHIC DATA OF PATIENT

1. **NAME:** _____
2. **SEX** **MALE** ☐ **FEMALE** ☐
3. **EDUCATION**

NO FORMAL EDUCATION ☐

STUDIED UPTO CLASS X ☐

STUDIED MORE THAN CLASS X ☐

5.OCCUPATION:

EMPLOYED ☐

UNEMPLOYED ☐

HOUSEWIFE ☐

STUDENT ☐

TOTAL HOUSEHOLD INCOME ☐

6.FAMILY TYPE:

NUCLEAR ☐

JOINT ☐

7. **FORMS OF TOBACCO USE**

SMOKING ☐

SMOKELESS ☐

BOTH ☐

8. **AGE OF INITIATION**_____
9. **AGE OF INITIATION DAILY**_____
10. **FREQUENCY OF TOBACCO**_____

ANNEXURE-3

Table 1. Fagerstrom Test of Nicotine Dependence (FTND)

Items	Responses	Scoring
1. How soon after you wake up do you smoke your first cigarette? *	Within 5 minutes	3
	6-30 minutes	2
	31-60 minutes	1
	After 60 minutes	0
2. Do you find it difficult to refrain from smoking in places where it is forbidden or not allowed?	Yes	1
	No	0
3. Which cigarette would you hate most to give up?	The first one in the AM	1
	All others	0
4. How many cigarettes per day do you smoke? *	10 or less	0
	11-20	1
	21-30	2
	31 or more	3
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?	Yes	1
	No	0
6. Do you smoke if you are so ill that you are in bed most of the day?	Yes	1
	No	0

Score 8+= high dependence

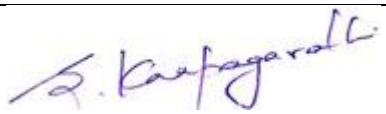
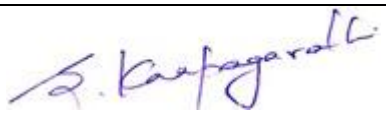
Score 5-7= moderate dependence

Score 3-4 = low to moderate dependence

Score 0-2 = low dependence

ANNEXURE-4

Questions	Answers	Points
How soon after you wake up to do you place your first dip? (min)	Within 5	3
	6–30	2
	31–60	1
	After 60	0
How often do you intentionally swallow tobacco juice?	Always	2
	Sometimes	1
	Never	0
Which chew would you hate most to give up most?	The first one in the morning	1
	Any other	0
How many cans/pouches do you chew per week?	More than 3	2
	2-3	1
	1	0
Do you chew more frequently during the first hour after awakening than during the rest of the day?	Yes	1
	No	0
Do you chew if you are so ill that you are in bed most of the day?	Yes	1
	No	0

11.	**SIGNATURE OF THE CANDIDATE**	
12.	**REMARKS OF THE GUIDE**	
10.	**10.1 NAME AND DESIGNATION OF GUIDE**	**DR. S. KARPAGAVALLI** PROFESSOR AND HEAD DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	10.2 NAME AND DESIGNATION OF Co- GUIDE	**DR. AVNISH SINGH** PROFESSOR HEAD OF THE DEPARTMENT DEPARTMENT OF PUBLIC HEALTH AND DENTISTRY
	10.3 SIGNATURE OF GUIDE	
	10.4 HEAD OF THE DEPARTMENT	**DR. S. KARPAGAVALLI** PROFESSOR AND HEAD DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	10.5 SIGNATURE	
11.	**11.1 REMARKS OF THE PRINCIPAL**	
	11.2 SIGNATURE	

PROFORMA FOR SHORT STUDY

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

SEEMA DENTAL COLLEGE AND HOSPITAL, RISHIKESH

"COMPARING EFFICACIES OF CLOTRIMAZOLE MOUTH PAINT WITH CLOTRIMAZOLE LOZENGES IN REDUCTION OF BURNING SENSATION IN PATIENTS WITH ORAL CANDIDIASIS."

GUIDE:

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SUBMITTED BY:

Dr. SHIVANI KAPURWAN

POST GRADUATE STUDENT

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2.	**COURSE OF STUDY & SUBJECT**	MASTER OF DENTAL SURGERY (M.D.S) IN ORAL MEDICINE AND RADIOLOGY.
3.	**DATE OF ADMISSION TO COURSE**	2nd NOVEMBER 2022
4.	**TITLE**	"COMPARING EFFICACIES OF CLOTRIMAZOLE MOUTH PAINT WITH CLOTRIMAZOLE LOZENGES IN REDUCTION OF BURNING SENSATION IN PATIENTS WITH ORAL CANDIDIASIS."

5	**BRIEF RESUME OF THE INTENDED WORK:** **5.1 Need for the study:** Fungi, being eukaryotic microorganisms, notably Candida species, are particularly relevant to dentistry due to their association with various oral infections. Among these, Candida albicans-induced candidiasis stands out as the most common type. Candida albicans constitutes a significant portion of the normal flora in the oral cavity, present in over 40% of individuals without symptoms. Factors such as aging and antibiotic usage have contributed to the rising prevalence of oral candidiasis. This condition is linked with both local factors like dry mouth, denture use as well as systemic risk factors including immune-suppressive conditions due to illness or medications.[2] Early identification of individuals at risk and proactive treatment for oral candidiasis are crucial to prevent severe complications and potentially fatal outcomes, especially in high-risk patients, such as the elderly wearing dentures.[1] The treatment options for thrush are somewhat limited, prompting a need to assess new therapeutic agents. Recently, clotrimazole, a novel synthetic antifungal drug, has entered clinical use. Clotrimazole, derived from imidazole, has primarily been employed locally to treat yeast and dermatophyte infections in the oral mucosa.[1] Initially, localized oral candidiasis should be managed with localized treatments targeting the affected area before resorting to systemic antifungal medications. While polyene antibiotics have traditionally been the first-line choice for antifungal treatment for almost fifty years, newer azoles such as ketoconazole has emerged as options for treating systemic fungal infections and clotrimazole have demonstrated efficacy in treating oral candidiasis.[2] Clotrimazole troches (CT) are topical antifungal agents recommended as a first-line treatment for mild oral candidiasis (OC) by the Infectious Diseases Society of America (IDSA) candidiasis clinical practice guidelines. Due to its low systemic drug concentrations, topical antifungal therapies result in fewer adverse events and a lower

	potential for drug-drug interactions, making them generally more preferred.[3] There is limited treatment options for oral candidiasis (thrush) thus prompt a need for new therapeutic approaches. Localized treatments like clotrimazole troches and clotrimazole mouth paint require evaluation for their effectiveness in managing burning sensation in oral candidiasis. Since no study has evaluated the comparison thus the aim of this study was to compare the efficacy of clotrimazole mouth paint with clotrimazole lozenges in the reduction of burning sensation in patients with oral candidiasis.

5.2 Aim:

The aim of this study is to compare the efficacy of clotrimazole mouth paint with clotrimazole lozenges in treating patients with burning sensation in oral Candidiasis patients

5.3: Objectives of the study:

1. To evaluate the efficacy of clotrimazole lozenges for relieving the burning sensation in patients with oral Candidiasis.
2. To evaluate and compare the effectiveness of clotrimazole mouth paint with clotrimazole lozenges in treating the burning sensation experienced by patients with oral Candidiasis.
3. To measure and compare the overall improvement in symptoms, such as burning reduction between the two treatment modalities.
4. To investigate any potential side effects or adverse reactions associated with the administration of clotrimazole mouth paint with clotrimazole lozenges.
5. To determine patient satisfaction and preference for either Clotrimazole Mouth paint and Clotrimazole Lozenges.

5.4: Review of Literature:

Rakefet Czerninski & Anna Pikovsky & Irith Gati & Michael Friedman & Doron Steinberg (2015)[4]

The objective of the study was to compare the efficacy of this varnish with clotrimazole troche treatment of oral candidiasis. In a study involving 12 patients diagnosed with denture stomatitis and treated over a span of 14 days, half were assigned to use Clot-SRV (referred to as the study group), while the other half used clotrimazole troches (referred to as the control group). Patients in the study group were instructed to apply Clot-SRV, containing 50 mg of clotrimazole, once daily, whereas those in the control group were

	directed to use five troches of 10 mg clotrimazole per day. Various microbiological samples were collected from saliva, buccal mucosa, palate, and denture surfaces. The severity of erythema was assessed at three different time points, and results suggest that the novel clotrimazole sustained release varnish could play a pivotal role in a revised protocol for managing oral candidiasis, potentially leading to enhanced clinical outcomes.
6	**Thamer A. Almangour et al (2021)[5]** In 2021 Thamer A. Almangour et al conducted a randomized clinical comparing clotrimazole with other antifungal agents in patients clinically diagnosed with oral candidiasis up to November 1st, 2019, four electronic databases, ongoing trial registries, and manual searches were utilized. Primary outcomes included clinical response and mycological cure rates, while secondary outcomes comprised relapse rate, systemic infection incidence, compliance, and adverse effects. They concluded that clotrimazole is less effective than fluconazole but comparable to other topical therapies for oral candidiasis treatment. **Pragati C Madane, A Sailaja Choudary, T A Deepak, M S Abhinethra, L Upasana, Ruthvik Balaji (2022)[6]** The study aimed to compare the clinical and mycological effectiveness of topical fluconazole and clotrimazole in the treatment of oral candidiasis. A total of 40 subjects were randomly allocated into two groups, each consisting of 20 individuals. Group 1 received topical clotrimazole treatment, while Group 2 received topical fluconazole treatment. Patients were graded based on severity, and swabs were taken for species identification and colony count. Treatment response was evaluated post-treatment for clinical signs and changes in colony count. Results showed a clinical resolution rate of 80% and 100% in the clotrimazole and fluconazole groups, respectively. The mycological

cure rate was 82.52% and 86.38% in the clotrimazole and fluconazole groups, respectively. In conclusion, fluconazole demonstrated a slightly superior clinical cure rate compared to clotrimazole, while the mycological cure rate was approximately similar for both treatments.

MATERIALS AND METHODS:

6.1 Source of data:

The patients to be studied will be selected from the outpatient Department of Oral Medicine & Radiology, Seema Dental College and Hospital, Rishikesh, Uttarakhand**.**

Study Design: Interventional

Study Period: March 2024 to June 2024

Place of study: Seema Dental College and Hospital, Rishikesh

Sampling design, method and size:

The sample size was calculated using the following formula based on the average proportion of improvement between the two groups.

$$N=\frac{2 \times P(1-P)(Z_{\alpha/2}+Z_{\beta})^2}{(P_1-P_2)^2}$$

where,

N= Sample Size

$Z_{\alpha/2}$= 1.44 [confidence interval of 85%]

Z_{β} = 0.842 [Power of study 80%]

P = 0.9 (pooled prevalence)

d = 0.2

So,

$$N = \frac{2 \times 0.9\,(0.1)\,(1.44 + 0.842]^2}{(0.2)^2}$$

$N = 23.4 \sim 24$

The minimum sample size required for the study is 48 (24 participants per group)

Inclusion criteria:

1. Participants willing to provide informed written consent for research participation
2. Patients presenting with clinically diagnosed oral candidiasis
3. Adults aged 14-50years.
4. Individuals with underlying systemic conditions

Exclusion criteria:

1. Participants not willing to provide informed written consent for research participation
2. Patients who are pregnant or breastfeeding
3. Patients with a known allergy to any components of the two treatment modalities
4. Patients had a known sensitivity to polyenes or the azole group of antimycotics
5. Patients unable to adhere to the prescribed treatment regimen or attend follow-up visits

Informed consent: Required

6.2 Method of collection of data

The present prospective study involves obtaining informed written consent for research participation.

Materials

The minimum sample size required for the study is 48 (24 participants per group)

Methodology

The patients will be divided into two groups: Group A will be treated with clotrimazole mouth paint and Group B will be treated with clotrimazole lozenges. Treatment will be administered both groups three times a day for 14 days. VAS will be recorded before treatment and at 7&14 days after the beginning of the treatment. The collected data will subsequently undergo statistical analysis

Statistical analysis:

The data will be analyzed using SPSS [ver 22.0]. Categorical data will be compared between the groups using the Chi-Square test. Data will be presented using graphs and tables in percentages. The level of significance will be set at $P < 0.05$.

.

6.3 Does the study require any investigation or interventions to be conducted on patients or other humans or animals?

Yes

6.4 Has ethical clearance been obtained from your institution in case of 6.3?

Yes

7	**List of references:**

1. *Yap BS, Bodey GP. Oropharyngeal candidiasis treated with a troche form of clotrimazole. Arch Intern Med. 1979 Jun;139(6):656-7.*

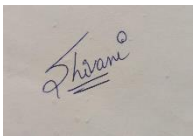
2. *Sholapurkar AA, Pai KM, Rao S. Comparison of efficacy of fluconazole mouthrinse and clotrimazole mouthpaint in the treatment of oral candidiasis. Aust Dent J. 2009 Dec;54(4):341-6. .*

3. *Vazquez JA, Patton LL, Epstein JB, Ramlachan P, Mitha I, Noveljic Z, Fourie J, Conway B, Lalla RV, Barasch A, Attali P; SMiLES Study Group. Randomized, comparative, double-blind, double-dummy, multicenter trial of miconazole buccal tablet and clotrimazole troches for the treatment of oropharyngeal candidiasis: study of miconazole Lauriad® efficacy and safety (SMiLES). HIV Clin Trials. 2010 Jul-Aug;11(4):186-96.*

4. *Czerninski R, Pikovsky A, Gati I, Friedman M, Steinberg D. Comparison of the efficacy of a novel sustained release clotrimazole varnish and clotrimazole troches for the treatment of oral candidiasis. Clin Oral Investig. 2015 Mar;19(2):467-73*

5. *Almangour TA, Kaye KS, Alessa M, Eljaaly K, Sfouq Aleanizy F, Alsharidi A, Al Majid FM, Alotaibi NH, Alzeer AA, Alnezary FS, Alhifany AA. Efficacy of clotrimazole for the management of oral candidiasis: A meta-analysis of randomized clinical trials. Saudi Pharm J. 2021 Apr;29(4):315-323.*

6. *Madane PC, Choudary AS, Deepak TA, Abhinethra MS, Upasana L, Balaji R. Comparative evaluation of fluconazole and clotrimazole in treatment of oral candidiasis. J Oral Maxillofac Pathol. 2022 Oct-Dec;26(4):595*

8.	**SIGNATURE OF THE CANDIDATE**	
9.	**REMARKS OF THE GUIDE**	
10.	**10.1 NAME AND DESIGNATION OF GUIDE**	**DR. S. KARPAGAVALLI** PROFESSOR AND HEAD DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	NAME AND DESIGNATION OF CO-GUIDE	**Dr. RANJEETA MEHTA** READER DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	10.2 SIGNATURE OF GUIDE	
	10.3 HEAD OF THE DEPARTMENT	**DR. S. KARPAGAVALLI** PROFESSOR AND HEAD DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	10.4 SIGNATURE	
11.	**11.1 REMARKS OF THE PRINCIPAL**	
	11.2 SIGNATURE	

PROFORMA FOR SHORT STUDY

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

SEEMA DENTAL COLLEGE AND HOSPITAL, RISHIKESH

"COMPARING EFFICACIES OF CURCUMIN MOUTHWASH WITH TETRACYCLINE MOUTHWASH IN ORAL ULCERS"

GUIDE:

Dr. S. KARPAGAVALLI
PROFESSOR
HEAD OF THE DEPARTMENT
DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
SEEMA DENTAL COLLEGE & HOSPITAL

CO-GUIDE:

Dr. RANJEETA MEHTA
READER
DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
SEEMA DENTAL COLLEGE & HOSPITAL

SUBMITTED BY:

Dr. ANSHUMAN JHA

2nd YEAR POST GRADUATE STUDENT

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

SEEMA DENTAL COLLEGE AND HOSPITAL, RISHIKESH

1.	**NAME OF THE CANDIDATE & ADDRESS** **(IN BLOCK LETTERS)**	**DR. ANSHUMAN JHA** 2nd YEAR POST GRADUATE STUDENT DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY SEEMA DENTAL COLLEGE AND HOSPITAL, VIRBHADRA ROAD, RISHIKESH-249203, UTTARAKHAND.
2.	**COURSE OF STUDY & SUBJECT**	MDS 2nd YEAR SUBJECT -ORAL MEDICINE AND RADIOLOGY.
3.	**DATE OF ADMISSION TO COURSE**	1ST DECEMBER 2022- 2025
4.	**TITLE**	COMPARING EFFICACIES OF CURCUMIN MOUTHWASH WITH TETRACYCLINE MOUTHWASH IN ORAL ULCERS

5	**BRIEF RESUME OF THE INTENDED WORK:** **5.1 Need for the study:** Recurrent aphthous stomatitis (RAS), colloquially known as Canker sores, ranks among the most prevalent oral mucosal afflictions. Notably, in India between 2010 and 2012, RAS exhibited a prevalence of 21.7%. This condition manifests acutely, primarily affecting the nonkeratinized oral mucosa. Clinically, RAS onset is heralded by a prodromal phase characterized by localized burning or pain enduring for 24 to 48 hours prior to ulceration.[1] Characteristically, RAS lesions manifest as self-limiting, painful ulcers, typically presenting as one to three shallow, round or oval-shaped ulcers, each featuring a shallow necrotic center. These ulcers are encased by a yellow-gray pseudomembrane, delineated by minimally raised margins and an erythematous halo, indicative of superficial vasculitis.[2] Both systemic and local tetracycline regimens have been enlisted in RAS management due to their documented antibacterial properties. Beyond their antimicrobial effects, tetracyclines have been found to attenuate collagen breakdown by inhibiting collagenase activity. Within the realm of Ayurveda, the traditional Indian system of holistic medicine, plant-derived remedies are predominantly utilized for treating diverse ailments, including cancer. Curcumin, the principal bioactive constituent of turmeric, has garnered centuries of recognition for its vivid yellow hue and robust pharmacological profile, including potent antioxidant, antiseptic, antibacterial, anti-inflammatory, immunomodulatory, and analgesic attributes. Its anti-inflammatory prowess is ascribed to its ability to impede the biosynthesis of inflammatory prostaglandins, thereby modulating cyclooxygenase and lipooxygenase activity, consequently hindering the release of prostaglandin leukotrienes and neutrophil function during inflammatory cascades.[3] Despite its therapeutic promise, comprehensive data delineating the correlation between pain scores and ulcer dimensions in RAS following topical curcumin and tetracycline application remains scarce. Hence, the present investigation endeavors to juxtapose the

efficacies of Curcumin and tetracycline mouthwashes in ameliorating Ulcer Severity Scores. Should parity be established, Curcumin may emerge as a preeminent therapeutic modality for RAS, considering the potential adverse effects associated with conventional allopathic interventions

5.2 Aim:

The present study aims to compare the efficacy of Turmwash Mouthwash with Tetracycline Mouthwash in treating patients with oral ulcers.

5.3: Objectives of the study:

1. To assess the effectiveness of Turmwash Mouthwash in treating oral ulcers.
2. To evaluate and compare the effectiveness of Turmwash Mouthwash with Tetracycline Mouthwash in the treatment of oral ulcers.
3. To measure and compare the overall improvement in symptoms, such as pain reduction and ulcer healing, between the two treatment modalities.
4. To investigate any potential side effects or adverse reactions associated with the administration of Turmwash Mouthwash and Tetracycline Mouthwash.
5. To determine patient satisfaction and preference for either Turmwash Mouthwash or Tetracycline Mouthwash in the management of oral ulcers.
6. To provide clinicians with evidence-based information that can guide treatment decisions for patients with oral ulcers.

5.4: Review of Literature:

Deshmukh RA and Bagewadi AS.[4] Conducted a randomized clinical trial in 2014 on 60 patients of either sex with clinically diagnosed minor RAS to assess and compare the efficacy of Curcumin with Triamcinolone acetonide in the gel form and concluded that no significant difference was noted in both the groups in the treatment of RAS.

Raman P and Pitty HR.[5] in 2020 conducted a study to assess and correlate pain score

with ulcer size using topical curcumin 2% gel and triamcinolone acetonide oral paste 0.12% in recurrent minor oral aphthous ulcerations. The study showed that Curcumin performed on par with triamcinolone and there was no positive correlation between ulcer size and pain score with topical management of Curcumin and Triamcinolone in aphthous ulcers.

Shamash MS and Zaidan TF[6] in 2020 inferred that the curcumin treatment reduced ulcer area from the 3rd day till the 7th day and improved ulcer healing at 14th day.

6 **MATERIALS AND METHODS:**

6.1 Source of data:

The patients to be studied will be selected from the outpatient Department of Oral Medicine & Radiology, Seema Dental College and Hospital, Rishikesh, Uttarakhand.

Study Design: Double Blinded Clinical Trial

Study Period: April 2024 to June 2024

Place of study: Seema Dental College and Hospital, Rishikesh

Sampling design, method and size:

The sample size was determined using the following formula:

$$N = \frac{2 \times (Z_{\alpha/2} + Z_{\beta})^2}{d^2}$$

where,

N= Sample Size

$Z_{\alpha/2}$ = 1.96 at 95% Confidence Interval [5% type I error].

Z_{β} = 1.04; Power of study 85%

d = 0.97 (effect size)

So,

$$N = \frac{2 \times (1.96 + 1.04)^2}{}$$

$(0.97)^2$

N= 19.1 (drop out of 10%)

N = 21.2 ~ 22

A minimum sample size of 22 participants in each group (2 x 22 = 44) will be required for the present study. Rounding off to 50 (25 in each group).

Inclusion criteria:

1. Participants willing to provide informed written consent for research participation
2. Patients presenting with clinically diagnosed oral ulcers.
3. Adults aged 14-50years.
4. Individuals with overall good general health, without significant comorbidities that could affect the study outcomes

Exclusion criteria:

1. Participants not willing to provide informed written consent for research participation
2. Patients who are pregnant or breastfeeding
3. Patients with a known allergy to any components of the two treatment modalities
4. Patients with severe systemic conditions (e.g., uncontrolled diabetes, immunodeficiency disorders) that may compromise the study results
5. Patients under treatment by immunosuppressive, chemotherapy, or immunomodulators
6. Patients unable to adhere to the prescribed treatment regimen or attend follow-up visits

Informed consent: Required

6.2 Method of collection of data

The present prospective study involves obtaining informed written consent for research participation. The patients will be divided into two groups: Group A will be treated with Turmwash Mouthwash and Group B will be treated with Tetracycline Mouthwash. Treatment will be administered both groups three times a day for 7 days

Materials

1. Turmwash Mouthwash
2. Tetracycline Mouthwash

Methodology

Ulcer Severity Score will be recorded before treatment and at 7 days after the beginning of the treatment. The collected data will subsequently undergo statistical analysis

Statistical analysis:

Data will be entered into the Excel sheet. Data will be analyzed using SPSS (Statistical Package for Social Sciences) 22.0 version, IBM Corp. Descriptive statistics will be performed. Categorical data will be compared using the Chi-Square test and continuous data between the groups will be compared using the unpaired 't' test or Mann-Whitney U test. The level of significance will be set at $P \leq 0.05$.

.

6.3 Does the study require any investigation or interventions to be conducted on patients or other humans or animals?

Yes

6.4 Has ethical clearance been obtained from your institution in case of 6.3?

Yes

7	**List of references:**

1. Altenburg A, El-Haj N, Micheli C, Puttkammer M, Abdel-Naser MB, Zouboulis CC. The treatment of chronic recurrent oral aphthous ulcers. Deutsches Ärzteblatt International. 2014 Oct;111(40):665.
2. Sharma D, Garg R. A comprehensive review on aphthous stomatitis, its types, management and treatment available. J Dev Drugs. 2018;7(2):1-8.
3. Shah S, Rath H, Sharma G, Senapati SN, Mishra E. Effectiveness of curcumin mouthwash on radiation-induced oral mucositis among head and neck cancer patients: A triple-blind, pilot randomised controlled trial. Indian Journal of Dental Research. 2020 Sep 1;31(5):718-27.
4. Deshmukh RA, Bagewadi AS. Comparison of effectiveness of curcumin with triamcinolone acetonide in the gel form in treatment of minor recurrent aphthous stomatitis: A randomized clinical trial. International journal of pharmaceutical investigation. 2014 Jul;4(3):138.
5. Raman P, Pitty HR. Correlation of pain score with ulcer size in oral aphthous ulcers using 2% curcumin gel and 0.1% triamcinolone oral paste-A parallel comparison study. Journal of Indian Academy of Oral Medicine and Radiology. 2021 Jan 1;33(1):53-9.
6. Shamash MS, Zaidan TF. Effect of topical curcumin on the healing of major oral mucosal ulceration. EurAsian Journal of BioSciences. 2020 Aug 1;14(2).

ANNEXURE-I

SEEMA DENTAL COLLEGE AND HOSPITAL

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

CONSENT FORM

I ……. Resident of ……. District ……. Uttarakhand give consent for my inclusion in the study titled "COMPARING EFFICACIES OF CURCUMIN MOUTHWASH (TURMWASH) WITH TETRACYCLINE MOUTHWASH IN ORAL ULCERS" conducted by Dr. Anshuman Jha under the guidance of Dr. S Karpagavalli, Professor and Head and Co-Guide, Dr. Ranjeeta Mehta, Reader in the Department of Oral Medicine and Radiology of Seema Dental College and Hospital.

Name Signature with Date

सीमा डेंटल कॉलेज एवं अस्पताल

मौखिक चिकित्सा एवं रेडियोलॉजी विभाग

सहमति पत्र

मैं …… निवासी ……. जिला …….. उत्तराखंड, "ऑरल अल्सर्स में कर्कुमिन माउथवॉश की प्रभावक्षमता की तुलना टेट्रासिक्लिन माउथवॉश के साथ" शीर्षक की अध्ययन में शामिल होने के लिए अनुमति देता हूँ जो सीमा डेंटल कॉलेज और अस्पताल के मौखिक चिकित्सा एवं रेडियोलॉजी विभाग के डॉ. अंशुमान झा द्वारा डॉ. एस कार्पगावल्ली, प्रोफेसर और प्रमुख और डॉ. रंजीता मेहता, विभाग के रीडर के मार्गदर्शन में किया जा रहा है।

नाम तिथि के साथ हस्ताक्षर

ANNEXURE-II

ULCER SEVERITY SCORE (USS)

Recurrent Aphthous Stomatitis

Name: Diagnosis:

Age/Sex: First visit to the department

OPD Number: Yes ☐ ☐ No

Date: Patient on medication for RAS

Oral Physician: ☐ Yes ☐ No

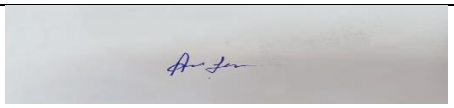
Medical History: Name of RAS medication:

Duration of medication:

	Ulcer characteristics	Score	Description of USS
Average **number** of ulcers			Score = average number of ulcers in a crop Maximum score = 20
Average **size** of ulcers (in mm)			Score = average size of ulcers in mm Maximum score = 20
Average **Duration** of ulcers (in weeks)			Score = number of ½ weeks i.e. Half a week (3 days) scores 1, one and a half week (10 days) scores 3. Maximum score = 10
Ulcer-free period (in weeks)			Score = 10 minus the average ulcer-free period in weeks Maximum score = 10 (never free from ulcers)
Pain as perceived by the patient (on a scale of 0–10)			1 for slight discomfort when ulcers are present.10 for excruciating ulcers interfering with eating, and talking Maximum score = 10
Mucosal **site**	**Group 1** Labial mucosa Buccal mucosa Buccal Sulcus Soft palate Ventral of tongue Floor of mouth **Group 2** Hard palate Attached gingiva Alveolar ridge Dorsum of tongue Tonsils Pillars of fauces Uvula Oropharynx		Score = total of sites affected 1 for each site in group 1 (non-keratinised mucosa) 2 for each site in group 2 (keratinised, specialized) Maximum score = 10

Evidence of scarring **Yes** ☐ **No** ☐

Total USS:

8	**SIGNATURE OF THE CANDIDATE**	
9	**REMARKS OF THE GUIDE**	
10.	**10.1 NAME AND DESIGNATION OF GUIDE**	**Dr. S. KARPAGAVALLI** PROFESSOR AND HEAD DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	NAME AND DESIGNATION OF CO-GUIDE	**Dr. RANJEETA MEHTA** READER DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	10.2 SIGNATURE OF GUIDE	
	10.3 HEAD OF THE DEPARTMENT	**Dr. S. KARPAGAVALLI** PROFESSOR AND HEAD DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY
	10.4 SIGNATURE	
11.	**11.1 REMARKS OF THE PRINCIPAL**	

	11.2 SIGNATURE	

Seema Dental College & Hospital

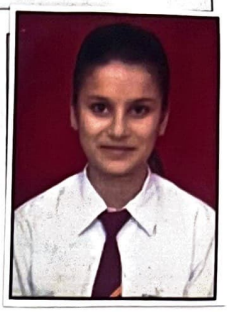
Virbhadra Road, Rishikesh- 249203,Uttarakhand

CERTIFICATE BY THE ETHICAL COMMITTEE

This is to certify that the **ICMR- STS 2023** Study entitled **"TO EVALUATE AND COMPARE THE ANTI-BACTERIAL AND ANTI-FUNGAL PROPERTIES OF CASSIA FISTULA EXTRACTS AT DIFFERENT PERCENTAGES INCORPORATED INTO ACRYLIC RESIN"** carried out by **Ms.RIYA RUPAM** of **BDS Batch 2020 with STS Reference ID: 2023-07744** was considered and cleared by the ethical committee on 23.09.2023

Dr. Jyotsna Seth
Member Secretary
Ethical Committee

STS 2023
REPORT ATTESTATION FORM (RAF)



(To be filled by the Student)

1. Name of the student : RIYA RUPAM
2. STS Reference ID : 2023-07744
3. Period of two months research done (Date-DD/MM/YYYY) : **From** 8th Oct'23 **Till** 8th Dec'24
4. Special research technique and methodology, if any, learnt : —
5. Personal impressions of the student about the STS program and what has the student gained from it : The program has been pretty intellectually stimulating I believe I have honed critical thinking skills & developed a nuanced perspective on complex skills

(To be filled by the Guide)

6. General Remarks of the Guide on Student's work & aptitude for research : Student was efficient & thoughtful in her management of the project. She is an eager learner & a quick study with deft technical ability.

UNDERTAKING

1. We, Riya Rupam (Name of Student) student of **(tick' appropriate)** MBBS/BDS I/II/III/IV year & Dr. Varun Kumar (Name of Guide) declare that we have abided by the Instructions and Terms & Conditions for STS given on ICMR website.
2. We had obtained necessary approvals from IEC/IAEC & Informed consent from participants (not applicable for animal studies).
3. We confirm that the data is our own and has not been plagiarized from any other source.
4. We have read all the Terms and Conditions for the STS-2023 report submission and we agree to all of them.
5. We are aware that ICMR reserves the right to reject the report/not release stipend/e-certificate without assigning any reason, if the report is not prepared/submitted as per given instructions/incomplete/ incorrect/ not as per objectives/ not as per ICMR format/ submitted late or any other technical errors/reason deemed suited by ICMR, if not submitted completely within the given deadline.
6. The information/research findings in report are true to best of our knowledge. We shall respect decision of ICMR.
7. We understand that scientific/personal details of other students/ guides/ copies of projects/titles/reviewers/ decision of reviewers/ minutes of meeting/ marks /scores *etc.* are confidential.
8. For presentation/publication of the scientific findings (oral/poster/abstract/paper) in any conference/ seminar/ meetings/ journals, due acknowledgement would be given to ICMR.
9. We understand that the e-certificate and stipend may be issued to the student only after approval of the report.

Signature of the Student Riya Rupam 8th Dec'24
(With Date)
Name of the Student RIYA RUPAM

Signature of the Guide 8/12/24
(With Date)
Name and Designation Dr. Varun Kumar
(With Seal)

Saveetha Dental College & Hospital
Hod Prosthodontics & Dental Material
Rishikesh

ICMR-STS 2023

REFERENCE ID:-2023-07744

TITLE

To Evaluate and Compare the Anti-Microbial and Anti-Fungal Properties of Cassia fistula Extracts at different percentages Incorporated into Acrylic Resin.

INTRODUCTION

INTRODUCTION

The microflora within our oral cavity consists of numerous essential microorganisms, playing a crucial role in safeguarding the human body against potential infectious threats. However, certain factors such as immunosuppression, malnutrition, inadequate oral hygiene, antibiotic misuse, trauma, and improper use of removable prostheses can elevate the likelihood of oral infections.[1] The oral cavity houses a myriad of bacteria, viruses, and fungi, with each individual hosting a distinct combination of these microorganisms. Candida albicans, a commensal microorganism, typically colonizes various regions of the human body, including the gastrointestinal tract, oral mucosa, and vagina.[2] Candidiasis, a prevalent fungal infection, is attributed to C. albicans. Notably, C. albicans, with its heightened virulence, is more commonly identified in clinical cases compared to the other 150 Candida species.[3]

Increased Candida colonization in the oral cavity is observed in individuals with weakened immune systems, thereby facilitating the onset of oral candidiasis. Moreover, factors such as dry mouth, smoking, oral prosthetics, dental caries, diabetes, and cancer therapy expedite the progression of this disease.[4] Candida albicans stands out as the leading cause of this infection, attributed to its capability to form biofilms and hyphae, as well as its production of hydrolytic enzymes and candialysin.[5] Despite the activation of mucosal defense, the creation of a hyphae-associated toxin by invading C. albicans cells results in a rise in the quantity and virulence of this pathogenic organism, culminating in infection.[6]

Although bacteria make up a substantial part of the oral microbiota, it's crucial to recognize the role of fungi, despite being a minor presence, they hold significance. Among these fungi, Candida species are notable as the primary colonizers in the oral cavity, adept at coexisting as commensals. Candida is uniformly dispersed in the mouth, with the tongue's dorsum being the most frequent site of isolation[7] . The establishment and stability of bacterial populations in the oral cavity are influenced by the generally benign behavior of many constituents of the human microbiota. In contrast, Candida's commensal relationship is a product of the host's robust innate and adaptive immune responses, which effectively restrain the growth of pathogens on the epithelial surfaces.[8]

The frequency of colonization increases among individuals with weakened immune systems, as well as those facing conditions such as cancer and the utilization of intra-oral devices like dentures and orthodontic appliances..[4] The oral cavity can act as a reservoir for yeast colonization of the gastrointestinal tract,

and yeast colonization, facilitated by saliva, can spread to other areas of the body. Rates of carriage increase during middle and later stages of life, with reported statistics indicating that 45% of neonates, 45–65% of healthy children, 30–45% of healthy adults, and up to 74% of older individuals carry Candida in their oral cavities. Given the opportunity, Candida species can trigger oral candidiasis, which primarily affects immunocompromised individuals and those with predisposing conditions, leading to the characterization of Candida infections as a "disease of the diseased."

Within the oral cavity, Candida overgrowth may precipitate discomfort, pain, altered gustatory sensation, dysphagia upon esophageal dissemination, masticatory and deglutition complications, consequently resulting in compromised nutritional intake.[9] In individuals with compromised immune function, dissemination of infection via the bloodstream or upper gastrointestinal tract can result in severe infection correlated with elevated morbidity and mortality rates. Systemic candidiasis can exhibit mortality rates of up to 79%. Therefore, it is imperative for preventative and therapeutic interventions to target the removal or mitigation of predisposing factors.

Dental caries stand as one of the most prevalent chronic conditions affecting nearly every child worldwide.[11] Staphylococcus aureus (Staph. Aureus), a gram-positive anaerobe, plays a crucial role in causing tooth decay by demineralizing teeth through the conversion of sugar into lactic acid.[12] Additionally, staph. aureus exhibits the ability to colonize dental surfaces.[13]

As Staphylococci have long been recognized as part of the oral flora; nevertheless, their influence on oral health and illness is still being debated.[14] The reported rates of isolating Staphylococcus aureus varied by group, ranging from 24% to 84% in healthy adult dentate oral cavities to 48% in those wearing dentures.[15] Furthermore, this bacteria has been linked to certain oral diseases such as angular cheilitis, parotitis, and staphylococcal mucositis. S. aureus may possibly have a role in dental implant failure, according to new research. In addition to infections produced by S. aureus in other areas of the body, some oral staphylococcal infections are likely the consequence of cross-infection from multiple sources.[16]

Furthermore, Escherichia coli, a gram-negative, facultative anaerobic, rod-shaped coliform bacterium, is commonly present in the distal portions of the intestine of endothermic organisms.[17] Though the majority of E. coli strains pose no threat, specific serotypes like EPEC and ETEC exhibit pathogenic characteristics, resulting in substantial foodborne illnesses in affected hosts.[18] Moreover, these strains can be associated with incidents of food contamination,

occasionally necessitating product recalls.[19] The vast majority of E. coli strains, on the other hand, are a natural component of the gut microbiota and are typically either non-harmful or even helpful to humans.[20] It should be noted that these non-pathogenic variants have gotten far less attention than their pathogenic relatives.

Recent progress, coupled with advancements in naturopathy, has introduced various plant-based extracts that enhance antibacterial and antifungal properties, contributing to improved oral health.

The objective of this investigation is to assess and evaluate the antibacterial activity and measure the zone of inhibition of extracts against a range of bacterial and fungal strains. This study has examined the microbiological efficacy of powdered extracts derived from Cassia fistula Linn. leaves, an ethnomedicinal plant, to determine their potential antibacterial effects against clinically relevant bacterial and fungal species. This study aims to investigate the antibacterial and antifungal properties of Cassia fistula Linn. leaves. The microbial efficacy of powdered leaf extracts from Cassia fistula Linn., an ethnomedicinal plant, are examined for potential antimicrobial effects against clinically relevant bacterial and fungal strains. The antibacterial and antifungal potentials of Cassia fistula extracts are assessed against Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative), and Candida albicans (fungal strain). Subsequent phytochemical analyses of the plants are conducted. The microbial efficacy of Cassia fistula is attributed to the presence of various secondary metabolites. Therefore, these plants hold promise for identifying bioactive natural compounds that could serve as leads in pharmaceutical research and development endeavors.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Sivanesan Karthikeyan , Kuppannan Gobianand (2010)[21] conducted a study that inferred , The antiulcer potential of ethanol leaf extract (ELE) derived from Cassia fistula Linn. (Caesalpinaceae) was examined using a gastric ulcer model induced by pylorus ligation. Rats received oral doses of ranitidine (30 mg/kg b.w.) or ELE at doses of 250, 500, and 750 mg/kg b.w. one hour before pyloric ligation. Assessment of gastric juice parameters four hours post-ligation revealed significant dose-dependent reductions in gastric volume, pH, free acidity, and total acidity following ELE administration, indicating potential antiulcer effects possibly mediated by reinforcing mucosal defense mechanisms. Moreover, ELE pretreatment maintained sialic acid and fucose levels while increasing hexose, hexosamine, total non-amino polysaccharide, total carbohydrate, and C:P ratio, suggesting preservation of the mucosal barrier. Additionally, ELE pretreatment attenuated lipid peroxidation and enhanced superoxide dismutase activity while reducing catalase activity, indicating scavenging of free radicals and antioxidant properties. These observations propose that the antiulcer efficacy of ELE, comparable to ranitidine, may stem from decreased gastric acid secretion, protection of the mucosal barrier, inhibition of free radical formation, and antioxidant effects.

Sujogya K Panda , L P Padhi (2011)[22] concluded after studying Cassia fistula Linn., a member of the Leguminosae family, is recognized as a medium-sized tree widely utilized in Ayurvedic medicine and traditional remedies for various common ailments. This literature review delves into sequential extraction methods, employing petroleum ether, chloroform, ethanol, methanol, and water solvents on the plant's leaves, aiming to assess their preliminary phytochemical composition and antibacterial properties. The results revealed notable inhibitory activity against Gram-positive bacteria across all extracts, with the ethanol extract demonstrating the highest efficacy. Minimum inhibitory concentrations ranged from 94 to 1500 µg/ml. The phytochemical analysis revealed the occurrence of alkaloids, flavonoids, carbohydrates, glycosides, proteins and amino acids, saponins, and triterpenoids, primarily in polar extracts (such as ethanol, methanol, and aqueous), in contrast to nonpolar extracts (petroleum ether and chloroform). Additionally, the ethanol extract underwent TLC bioautography and a time-kill

study against Staphylococcus epidermidis, providing additional evidence of its broad-spectrum antibacterial properties.

These findings collectively suggest the potential of Cassia fistula leaf extracts in combating infectious diseases, indicating avenues for their utilization in therapeutic interventions.

N R Bhalodia , V J Shukla (2011)[23] conducted a study about the antibacterial and antifungal properties of Cassia fistula Linn. leaves. The objective was to evaluate the antibacterial activity and inhibitory zones of extracts against a variety of bacterial and fungal species. Hydroalcoholic extracts from Cassia fistula Linn. leaves were examined for their antimicrobial effectiveness against clinically relevant bacterial strains (Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa) and fungal strains (Aspergillus niger, Aspergillus clavatus, Candida albicans). The agar disc diffusion method was employed to assess the antibacterial activity of the extracts at concentrations ranging from 5 to 250 g/ml. The zone of inhibition produced by Cassia fistula extracts was compared with standard antibiotics such as ampicillin, ciprofloxacin, norfloxacin, and levofloxacin.

Irshad , Sheikh Shreaz, et al. (2011)[24] concluded from their study that In the realm of folk medicine, Cassia fistula Linn. (Caesalpiniaceae) has long been esteemed. Notably, the fruit of this plant harbors rhein, an anthraquinone derivative recognized for its antimicrobial prowess. Despite this, investigations into its potential as an anticandidal agent have been notably absent. This study endeavors to elucidate the phytochemical composition of both the fruit pulp and seed extracts of Cassia fistula, as well as their impact on Candida albicans ATCC 10261, Candida glabrata ATCC 90030, and Candida tropicalis ATCC 750. Methodologies employed involved standard phytochemical screening methods, with rhein identification accomplished through thin-layer chromatography. The anticandidal potential was assessed through a battery of assays, including minimum inhibitory concentration (MIC) determination, growth curve studies, cytotoxicity evaluations, and ergosterol estimation. Results underscored the substantial phenolic compound content within the fruit pulp and seed extracts. Rhein, a key constituent, was notably present in both extracts. MIC values varied, with the fruit pulp extract demonstrating heightened efficacy compared to the seed extract against the tested Candida strains.

Ergosterol, a vital component of fungal cell membranes, exhibited marked reduction upon treatment with the extracts, with fluconazole serving as a comparative benchmark. Discussion centers on the observed correlations between MICs, cytotoxicity profiles, and ergosterol inhibition, bolstering the assertion of Cassia fistula fruit pulp and seed extract as viable sources of anticandidal agents. This study underscores the promising potential of crude extracts from Cassia fistula as candidates for further exploration in the quest for novel anticandidal compounds.

A R Sunil , K K Amithamol,et al (2013)[25] conducted a study about the acaricidal efficacy of crude ethanolic extract from Cassia fistula leaves against Rhipicephalus (Boophilus) annulatus through the adult immersion test (AIT). Various concentrations of the extract (ranging from 50 to 100 mg/ml) were evaluated for their effects on adult mortality, fecundity inhibition, and egg hatching. Results were statistically analyzed using one-way ANOVA. Notably, concentrations above 80 mg/ml of the extract led to complete inhibition of egg hatching. The mortality of adult engorged female ticks and fecundity inhibition demonstrated concentration-dependent responses. The calculated LC50 value for the extract against R. (B.) annulatus was determined to be 97.1 mg/ml. These findings underscore the potential of Cassia fistula leaf extract as a promising acaricidal agent against R. (B.) annulatus, highlighting its concentration-dependent effects on tick mortality and reproductive processes.

Luma M Al-Nema , Reem N. Al-Irhayim et al (2014)[26] concluded that the effects of three different natural medicinal plant oils on the transverse strength, residual monomer levels, and hardness of polymethylmethacrylate (PMMA). A total of 105 samples were prepared, comprising 15 control samples of heat-cured ProBase acrylic resin without additives, and 90 samples with additives (ginger oil, meramia, and eucalyptus oil) at two concentrations (1.5 and 2.5). Transverse strength, indentation hardness, and residual monomer levels were assessed. The findings revealed a notable contrast in transverse strength and hardness between the control and additive groups, with oil additives diminishing transverse strength and elevating hardness. Although residual monomer levels did not significantly differ between the control and additive groups, the control group initially exhibited higher residual monomer release. In summary, the inclusion of eucalyptus, meramia, and ginger oils in acrylic resin led to reduced transverse strength, increased hardness, and no significant difference in residual monomer release.

Débora P. Antunes, Ana Carolina (2014)[27] concluded that the The influence of green tea aqueous extract and alcohol-free oral antiseptic on Candida albicans biofilm formation on heat-curing acrylic resin plates was examined. Candida is associated with oral candidiasis in inadequately cleaned dentures. Standardized heat-cured (Conv; n = 30) or microwave-cured acrylic resin (Mw; n = 30) specimens were segmented into six groups:

Conv resin with green tea extract, Conv resin with mouthwash, Conv resin control, Mw resin with green tea extract, Mw resin with mouthwash, and Mw resin control. After contamination with C. albicans, specimens were treated with the extract or mouthwash for 15 minutes. The results showed significant reductions in fungal cell counts for Conv resin with green tea extract (33.65%) and mouthwash (17.06%) compared to control (100%). Similarly, for Mw resin, mouthwash (43.16%) significantly reduced cell counts compared to control (100%). The study concludes that green tea extract and mouthwash effectively reduced viable fungal cells in acrylic resin biofilms.

M G McCormack, A J Smith et al. (2015)[16] conducted a study about the involvement of intraoral Staphylococcus aureus in disease and its role as a potential source of cross-infection is a subject of controversy. The study provides a 10-year retrospective analysis of laboratory data that reports the isolation of S. aureus from clinical specimens obtained from the oral and perioral regions. A compilation and analysis of laboratory records were conducted, focusing on specimens in which Staphylococcus aureus was isolated. The data spanned from January 1998 to December 2007 and originated from the Oral Microbiology Laboratory at Glasgow Dental Hospital. During the study duration, a total of 11,312 specimens were submitted to the laboratory. Staphylococcus aureus was identified in 1,986 specimens, constituting 18% of the total. Among these, 1,782 (90%) were methicillin-sensitive S. aureus (MSSA), while 204 (10%) were methicillin-resistant S. aureus (MRSA). Oral rinse samples were the most common source of MSSA isolations, whereas tongue swabs were predominant for MRSA. The majority of MRSA isolates belonged to the EMRSA-15 or EMRSA-16 lineages. These results indicate that Staphylococcus aureus remains commonly identified in the oral cavity and perioral region. The oral cavity should be recognized as a potential source of S. aureus for cross-infection and dissemination to other areas of the body. Additionally, the involvement of S. aureus in the pathogenesis of specific oral diseases should be considered as part of the differential diagnosis.

N. L. Martins Almeida , Luiz Leonardo Saldanha et al (2017)[28] evaluated Equisetum giganteum and Punica granatum fractions added to denture adhesive for their antimicrobial effects against C. albicans biofilm. Biofilms formed on heat-cured acrylic resin were treated with adhesive/herb extract mixtures, and antimicrobial activity was assessed. Both herb extracts enhanced the adhesive's anti-biofilm action on acrylic resin for up to 12 hours. Combining these extracts with COREGA® showed promise in biofilm control, offering temporary solutions for Denture Stomatitis treatment and prevention.

K.N.Okeke, Anisa Vahed et al. (2017)[29] conducted a study about the Several materials have been investigated to bolster the strength and fatigue resistance of poly methyl methacrylate (PMMA) denture base resins, but scant evidence exists regarding the utilization of natural fibers. This study aimed to evaluate the flexural and impact strengths of PMMA acrylic resins reinforced with Hibiscus sabdariffa fibers. Out of 50 fabricated PMMA specimens, 40 were fortified with varying weight percentages (wt%) of Hibiscus sabdariffa fibers, while 10 remained unfortified. The specimens were segregated into two groups, each containing 25 samples, for flexural and impact strength assessment.

Statistical analysis using ANOVA and Bonferroni tests revealed significant differences among the groups. Flexural strength results indicated that PMMA reinforced with 7.5 wt% fibers exhibited the highest mean value, followed by 10 wt%, unreinforced, 5 wt%, and 2.5 wt% reinforcement. Similarly, impact test results showed that 7.5 wt% reinforcement led to the highest mean value, indicating an improvement in the strength properties of PMMA denture base resins.

K. Vikash (2018)[30] evaluated the challenges faced by denture soft lining materials due to Candida albicans colonization, which can contribute to denture stomatitis. In an effort to mitigate this issue, the study investigated the effects of incorporating Aloe vera powder into heat-cured acrylic soft-liner powder on Candida albicans adherence, shear bond strength, and tear strength. Two concentrations of Aloe vera powder (3% and 10%) were tested based on preliminary findings. The assessments included tests for Candida adherence, shear bond strength, and tear strength, with longer-term effects evaluated after 2 and 4 weeks in artificial saliva. Statistical analysis using SPSS software indicated significant reductions in Candida albicans count with both concentrations of Aloe vera, accompanied by increased shear bond strength. However, tear strength did not show significant differences. Following incubation, all experimental groups displayed decreased Candida albicans count and improved shear bond and tear

strength, underscoring the potential of Aloe vera in augmenting the anti-Candida properties and mechanical strength of soft liners.

A.R.Abdulwahhab , R.K.Jassim (2018)[31] concluded that denture soft lining materials often face issues with Candida albicans colonization, potentially leading to denture stomatitis. To address this, the study examined the impact of incorporating Aloe vera powder into heat-cured acrylic soft-liner powder on Candida albicans adherence, shear bond strength, and tear strength. Two concentrations (3% and 10%) of Aloe vera powder were tested based on pilot study results. Tests included Candida adherence, shear bond strength, and tear strength, with long-term effects assessed after 2 and 4 weeks in artificial saliva. Statistical analysis using SPSS software revealed significant reductions in Candida albicans count with both Aloe vera concentrations, along with increased shear bond strength. Tear strength showed non-significant differences. After incubation, all experimental groups exhibited decreased Candida albicans count and increased shear bond and tear strength, highlighting the potential of Aloe vera in enhancing the anti-Candida properties and mechanical strength of soft liners.

P Sony , M Kalyani et al. (2018)[32] investigated the potential of Cassia fistula's leaves, bark, and seeds as agents against fluconazole-resistant Candida strains isolated from HIV patients was investigated, with a focus on identifying the key phytochemical constituents responsible for their fungicidal activity. Various extracts from Cassia fistula were evaluated against both Microbial Type Culture Collection (MTCC) Candida strains and fluconazole-resistant clinical isolates using agar diffusion and broth dilution methods. High-performance thin-layer chromatography was utilized to isolate the active phytochemical component in the ethanol seed extract, followed by docking studies to assess its binding affinity with lanosterol 14-alpha demethylase, a crucial azole drug target. The findings revealed significant anticandidal activity across all Cassia fistula extracts, with the ethanol seed extract demonstrating the most potent inhibitory effects. Notably, gallic acid emerged as the primary active phytochemical component, displaying robust binding affinity with lanosterol 14-alpha demethylase. This study underscores the potential of Cassia fistula extracts, particularly gallic acid, as promising natural antifungal agents against fluconazole-resistant Candida strains, emphasizing the need for further exploration of their pharmacokinetic properties.

Ban Nahal Shukur , Sahar Manfi Ahmed et al. (2019)[33] concluded that the efficacy of tea tree oil as an additive to heat-cured acrylic resin in combating Candida albicans. Twenty-four samples were prepared without oil (control), and 24 with 20% tea tree oil. These disks were inoculated with C. albicans and rinsed with saline to remove loosely attached cells. Colony-forming units (CFU) were measured on Sabouraud's dextrose agar plates. Control and treated disks were immersed in water for 1, 21, and 42 days and cleaned daily. Results showed that CFU for control disks decreased from 1.5000 to 1.0000 after 42 days, while those with tea tree oil decreased from 0.8750 to 0.3750. Tea tree oil-incorporated specimens effectively reduced C. albicans growth after 42 days. This indicates that tea tree oil could serve as a viable oral topical remedy for denture stomatitis by impeding the growth of C. albicans in heat-cured acrylic resin. In general, the research underscores the encouraging antimicrobial attributes of tea tree oil in dental contexts, presenting a fresh approach to addressing oral infections linked to denture usage.

Rihem Chaaben, Rym Taktak,et al. (2020)[34] concluded that a new biocomposite incorporating Salvadora persica powders into poly(methyl methacrylate) resin was developed, aiming to enhance the bioactive properties of dental restorative materials. This study marks the first instance of utilizing S. persica for this purpose. Material characterization involved both the base materials and the newly formulated biocomposite (with 30 wt% of S. persica). Techniques such as X-ray diffraction, Fourier transform infrared spectroscopy, differential scanning calorimetry, and high-performance liquid chromatography were employed for this purpose. Results from the dental material analysis revealed the presence of organic chemical compounds from S. persica responsible for its biological activities, along with mineral chemical compounds beneficial for dental applications and health. Importantly, no toxic residual monomers were detected. Furthermore, the biocomposite demonstrated antioxidant properties, as evidenced by its total polyphenol flavonoid content, and displayed antibacterial activity attributable to S. persica.

Z.S.Mawlood, A.H.Naji (2021)[35]- conducted a study to investigate The influence of Bergamot Essential Oil (BEO) on the transverse strength, impact strength, and surface roughness of heat-cured acrylic denture bases, crucial for fracture resistance and durability, was investigated. Ninety samples were categorized into groups with varying concentrations of BEO (0%, 5%, and 6% by volume) added to heat-cured acrylic resin. Assessments encompassed transverse strength, impact strength, and surface roughness. Statistical analysis of the data was conducted

using One-Way ANOVA, Tukey HSD, and Dunnett T3 tests. Incorporating 5% and 6% BEO resulted in a significant increase in transverse strength ($p < 0.000$), while impact strength remained unaffected. Surface roughness significantly decreased with 5% and 6% BEO compared to the control group. Bergamot oil integration improved transverse strength and surface smoothness without compromising impact strength, suggesting its potential as a beneficial additive in heat-cured acrylic denture bases.

Steve An, Jane L. Evans et al. (2021)[36] concluded that denture base resins (DBRs), like polymethyl methacrylate, are extensively used in removable denture fabrication due to their physical, mechanical, and esthetic attributes. However, they often serve as substrates for microorganism adherence, leading to biofilm formation and potential oral complications. This issue is particularly critical in geriatric and immunosuppressed patients. Thus, enhancing the antimicrobial properties of DBRs is crucial. This systematic review aimed to evaluate literature on the antimicrobial activity of DBRs incorporating antimicrobial agents or materials. A search of English peer-reviewed literature up to February 2019 was conducted using various databases. Twenty-eight articles met the inclusion criteria, revealing antimicrobial materials categorized into antimicrobial monomers or copolymers, phytochemical or phytomedical components, and other compounds. Strategies for incorporating these substances into DBRs and their impact on microbial growth reduction were identified. Despite efforts to improve DBRs' antimicrobial ability, this review found inconclusive evidence regarding the effectiveness of incorporating antimicrobial agents into DBRs.

Ban Nahal Shukur (2021)[37] conducted an investigation to examine how tea tree oil influences the impact strength, transverse strength, and hardness of heat-cured acrylic resin. A total of 80 specimens were fabricated, with 20 serving as controls and 60 incorporating tea tree oil at concentrations of 10%, 15%, and 20%. The results demonstrated notable distinctions between the control and experimental groups in both transverse strength and hardness assessments. However, no notable disparity was found in impact strength between the control and tea tree oil addition groups. Tea tree oil addition reduced hardness across all concentrations but enhanced transverse strength notably at the 20% concentration. Incorporating 20% tea tree oil into acrylic resin decreased hardness, improved transverse strength, and did not significantly affect impact strength.

Montri Ratanajanchai , Widchaya Kanchanavasita et al. (2021)[38] conducted a study to address the issue of microbial colonization on denture bases by introducing three food preservatives, zinc oxide, potassium sorbate, and sodium metabisulfite, into heat-polymerized poly(methyl methacrylate) (PMMA). The modified PMMA resins were assessed for their efficacy against Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans, as well as their cytotoxicity on mouse fibroblast L929 cells. Additionally, flexural strength and modulus properties were evaluated through a three-point flexural test. Results indicated that the incorporation of all preservative agents reduced microbial growth, with PMMA combined with sodium metabisulfite showing the highest antimicrobial activity. Furthermore, the modified resins showed no significant cytotoxicity. While the addition of food preservatives did not significantly affect flexural strength, it did decrease the flexural modulus of PMMA. Despite these alterations in mechanical properties, the materials still maintained acceptable flexural characteristics, suggesting that these food preservatives, particularly sodium metabisulfite, could serve as effective antimicrobial additives in denture base resins.

S. O. Bajunaid (2022)[39] aimed to study that Denture stomatitis, caused by Candida albicans, is a prevalent infection under removable dentures, posing systemic candida infection risks if untreated. Various treatments exist, yet none have proven entirely effective. This study aims to assess novel techniques integrating antimicrobial and protein-repellent agents into denture acrylic resin materials. A systematic review examined electronic databases for relevant studies . Results indicated significant antimicrobial activity with minimal impact on physical and mechanical properties, though optical properties were notably affected at higher concentrations. Incorporating antimicrobial agents effectively reduced Candida albicans biofilm formation on acrylic resin, with efficacy proportional to agent concentration. Careful consideration is warranted to preserve the denture base material's physical, mechanical, and optical properties.

Abeer A. Noori, Makarem A. Jaber (2022)[40] investigated the influence of integrating Neem or Aloe Vera on the thermal conductivity and shear bond strength of heat-cured acrylic soft liners was investigated, targeting their typical limitations of inadequate thermal conductivity and bonding with denture bases. Sixty specimens were categorized into groups for evaluating these properties through respective tests. Each major group was further subdivided into control, Neem, and Aloe Vera groups. Thermal conductivity specimens were fabricated with specific dimensions, while shear bond strength specimens consisted of

acrylic blocks with reline material applied between them. Thermal conductivity was measured using the Lee disc equipment, and shear bond strength was assessed with a specified crosshead speed. Statistical analysis was performed using One-way ANOVA and LSD tests. Results indicated that the Neem group exhibited the highest mean values for both thermal conductivity and shear bond strength compared to the Aloe Vera and control groups, with a significance level of $p<0.01$. Incorporating either Neem or Aloe Vera showed promising improvements in the thermal conductivity and shear bond strength of the soft liner material.

Ajay Singh Kushwah , Roopal Mittal et al. (2022)[41] investigated Cassia fistula bark's potential to protect against isoproterenol-induced cardiotoxicity in rats. Sequential extraction with five solvents yielded bark extracts, followed by in vitro antioxidant evaluations. Oral toxicity tests preceded myocardial studies in rats subjected to isoproterenol for two days. Methanolic extract (CFME) was administered at two doses: CFME-LD (250 mg/kg) and CFME-HD (500 mg/kg). CFME notably decreased lipid peroxidation, elevated antioxidant levels, and mitigated triglyceride and cholesterol levels. Additionally, it reduced ALT and AST activity in serum and exhibited protective effects against myocardial infarction. This study underscores CFME's antioxidative and cardioprotective attributes in counteracting

A P Godovalov , I I Zadorina et al. (2022)[42] conducted a study about An expedited detection method for Escherichia coli and bacteria belonging to the Escherichia coli taxonomic group within the oral microbiota. The study encompassed 44 participants, from whom specimens were gathered from various oral sites, including oral fluids (n=11), gingival fluids (n=11), oral mucosa smears/prints (n=11), and dental biofilm (n=11). These specimens were cultured in Koda's medium, and following a 24-hour incubation period, the examination focused on alterations in color and clarity. The maintenance of the initial green hue and clarity signified the absence of E. coli and Escherichia coli group bacteria in the sample. Conversely, a transition in the medium's color to yellow, cloudiness, and/or the formation of bubbles indicated the presence of E. coli and associated bacteria from the same group.

Concurrently, the collected samples were inoculated onto Endo agar, and the strains were identified to the species level. The study revealed complete agreement between the outcomes obtained via the traditional bacteriological approach and the utilization of Koda's medium. The latter presented a notable advantage in terms of result turnaround time (18-20 hours), compared to the

conventional method, which requires interpretation only after 72 hours or more. This corresponds with the prevailing norms in clinical microbiology and rapid diagnosis, adhering to the "point-of-care testing/doctor's office" diagnostic principle. The proposed technique shows potential for effective implementation in clinical settings for the localized diagnosis of E. coli and bacteria from the Escherichia coli group in the oral cavity.

S. S. Al-Shammari, F. M. Abdul-Ameer (2023)[43] concluded that Polymethylmethacrylate (PMMA) serves as a model for various dental materials, although its mechanical properties are not optimal. To address this, different fillers and oils like bergamot essential oil, thymol, eucalyptus, mermaid, and ginger have been explored. the influence of adding lemongrass essential oil (LGEO) to heat-cured PMMA denture base material and its impact on PMMA characteristics. The methodology involved the preparation of 120 samples divided into four groups corresponding to specific tests (transverse strength, impact strength, surface roughness, and surface hardness). Each group was further subdivided into control (0 vol.% additive) and two experimental groups (with 2.5 vol.% LGEO and 5 vol.% LGEO). Findings indicate that the inclusion of 5 vol.% LGEO significantly enhances mechanical and physical properties, including transverse strength, impact strength, and surface roughness. While 2.5 vol.% LGEO has a lesser effect, particularly on surface hardness. Incorporating LGEO enhances the overall mechanical and physical properties of heat-cured acrylic material.

Hummudi, G. Faisal et al (2023)[44] concluded that in recent times, herbal treatment has gained validation as a secure and efficient substitute for antimicrobial drugs, given its safety and effectiveness. The root extract of Eurycoma longifolia, also known as jack root extract (E.L.), has been acknowledged for its documented antibacterial and antifungal properties. Acrylic resin is commonly used in the production of dentures; however, its porous nature makes it susceptible to Candida albicans adhesion and infection. The objective of this study was to assess the impact of incorporating E.L. root extract into acrylic resin on the characteristics of heat-polymerized denture material. The research findings indicate that the incorporation of Eurycoma longifolia root extract into heat-cure acrylic resin resulted in improved surface hardness without causing any changes in color or surface roughness. This suggests that E. longifolia root extract can serve as a natural and safe antimicrobial agent when integrated into the resin.

F. Golfeshan, S. A. Mosaddad, et al. (2023)[45] aimed to study that in this in-vitro investigation, the antimicrobial impact of integrating hydroalcoholic extract from Punica granatum (P. granatum) into self-curing acrylic resins was evaluated against Streptococcus mutans (S. mutans) and Candida albicans (C. albicans).
In this study,the flowers of pomegranate (P. granatum) were crushed, and the hydroalcoholic extract was subsequently prepared. Serial dilutions (4-512 mg/mL) of the extract were made, and acrylic discs were created by blending 500 mg of acrylic resin powder with 0.3 mL of liquid monomer and 0.15 mL of the extract for each disc. Subsequently, both experimental and control discs were sterilized via autoclaving, and their impact on S. mutans and C. albicans was evaluated through a direct contact test and colony count. Data analysis was conducted using Kruskal-Wallis and Mann-Whitney tests. Upon the testing and investigations, the extract did not exhibit a significant reduction in the number of C. albicans colonies at any concentration. However, it demonstrated a concentration-dependent decrease in the number of S. mutans colonies. Pairwise comparisons indicated significant differences between the control group and concentrations of 512 mg/mL, 256 mg/mL, and 128 mg/mL, as well as between concentrations of 512 mg/mL and 256 mg/mL in comparison to the 4 mg/mL concentration. Additionally, significant differences were observed between concentrations of 512 mg/mL and 8 mg/mL in the S. mutans colony count. Notably, the concentration of 128 mg/mL represented the minimum threshold showing a significant difference from the control group in terms of antibacterial activity.

Seng Chiew Toh , Samuel Lihan et al (2023)[46] concluded that the comprehensive extraction and screening process of Cassia alata Linn., encompassing maceration and Soxhlet extraction techniques across leaves, roots, and stems using four solvents: n-hexane, ethyl acetate, ethanol, and distilled water. The crude extracts underwent rigorous evaluation employing agar well diffusion, colorimetric broth microdilution, grid culture, and bacterial growth curve analysis against Staphylococcus aureus. Identification of phytochemicals within the crude extracts was facilitated by Gas Chromatography-Mass Spectrometry (GC-MS). Results unveiled ethyl acetate extraction as particularly efficacious, demonstrating the largest inhibition zones and lowest minimum inhibitory concentrations against S. aureus. Notably, treatment with the crude extract prolonged the lag phase of bacterial growth, indicating significant antimicrobial activity. GC-MS analysis identified 88 phytochemicals, including fatty acids, esters, alkanes, phenols, fatty alcohols, sesquiterpenoids, and macrocycles, with 32 previously recognized for their antimicrobial, antioxidant, and anti-inflammatory properties. Conclusively, the ethyl acetate crude extract displayed superior antimicrobial efficacy, particularly from the roots and stems

of Cassia alata. However, further exploration of the remaining 56 phytochemicals is warranted to unlock their full medicinal potential.

Hummudi, G. Faisal et al (2023)[47] aimed at studying Eurycoma longifolia root jack extract (E.L.) is recognized for its antibacterial and antifungal properties. Acrylic resin, used in denture production, can harbor Candida albicans, leading to infections. This study aimed to assess the effects of adding E.L. root extract to acrylic resin on denture material properties. Sixty specimens were prepared from heat-polymerized acrylic resin and divided into control and experimental groups. E.L. root extract was incorporated into the experimental groups during resin preparation. Surface hardness, roughness, and color changes were evaluated. Results showed enhanced hardness in the experimental group, with no significant changes in roughness. Color analysis revealed no significant alterations except for the blue color. Overall, E.L. root extract exhibited potential as a natural antimicrobial agent without adversely affecting acrylic resin properties.

AIM AND OBJECTIVES

AIM AND OBJECTIVES OF THE STUDY

AIM-

To Evaluate and Compare the Anti-Microbial and Anti-fungal properties of Cassia Fistula extracts at different percentages incorporated into Acrylic Resin

OBJECTIVES-

1. To evaluate and compare the properties of altered acrylic resin by the addition of leaf extract of Cassia fistula Linn. against Gram Positive Bacteria- Staphylococcus aureus against conventional acrylic resin.
2. To evaluate and compare the properties of altered acrylic resin by the addition of leaf extract of Cassia fistula Linn. against Gram Negative Bacteria- Escherichia coli against conventional acrylic resin.
3. To evaluate and compare the properties of altered acrylic resin by the addition of leaf extract of Cassia fistula Linn. against Fungi- Candida albicans against conventional acrylic resin.

MATERIALS AND METHOD

MATERIAL AND METHOD

Materials-

1. Cassia fistula Linn. Extracts (Amaltas Extracts)
2. Heat-cure acrylic material (DPI)
3. Heat-cure monomer (DPI)
4. Cold-mould seal (DPI)
5. Escherichia coli strain
6. Candida albicans strain
7. Staphylococcus aureus strain
8. Plaster of Paris
9. Vaseline

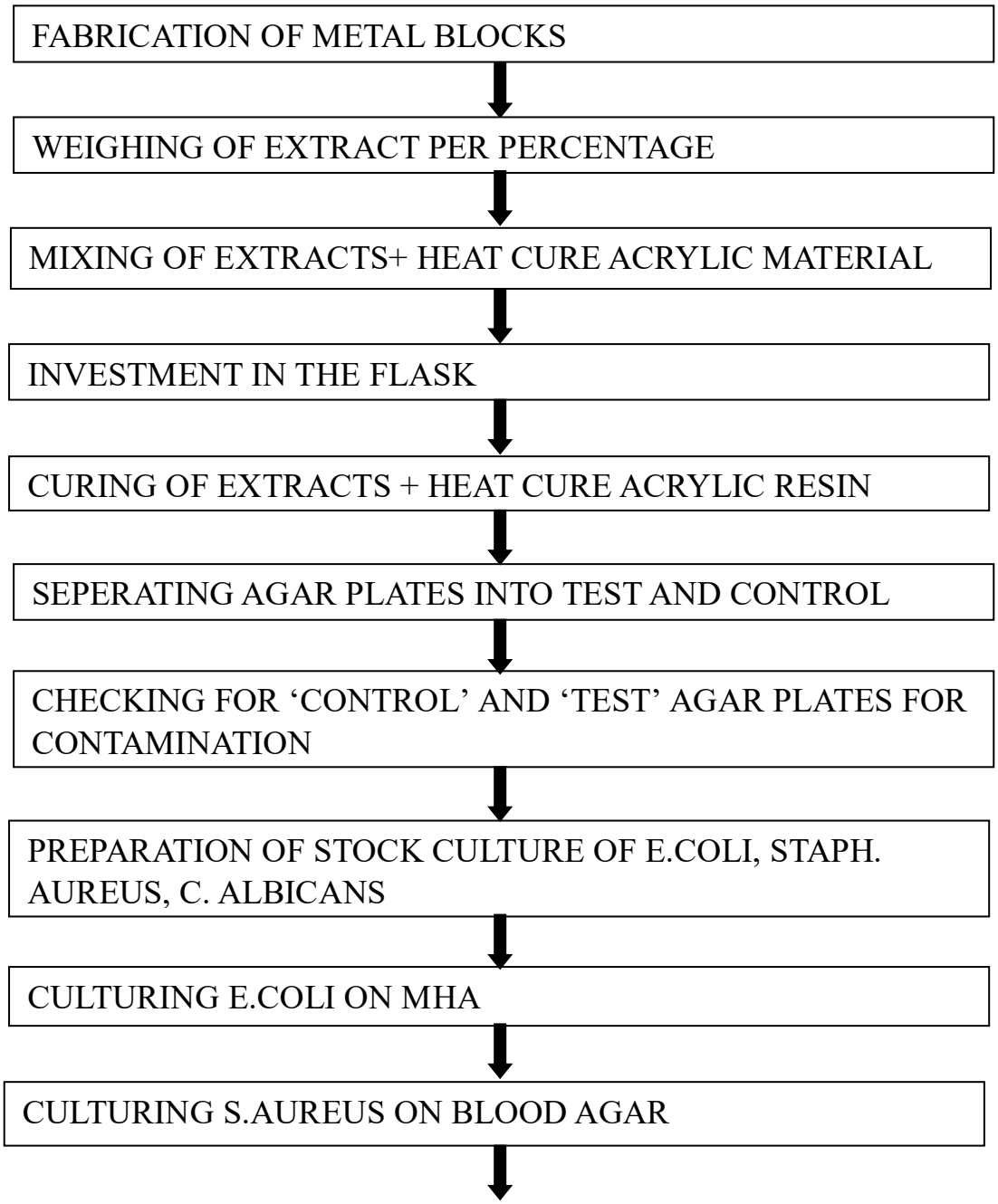
Armamentarium-

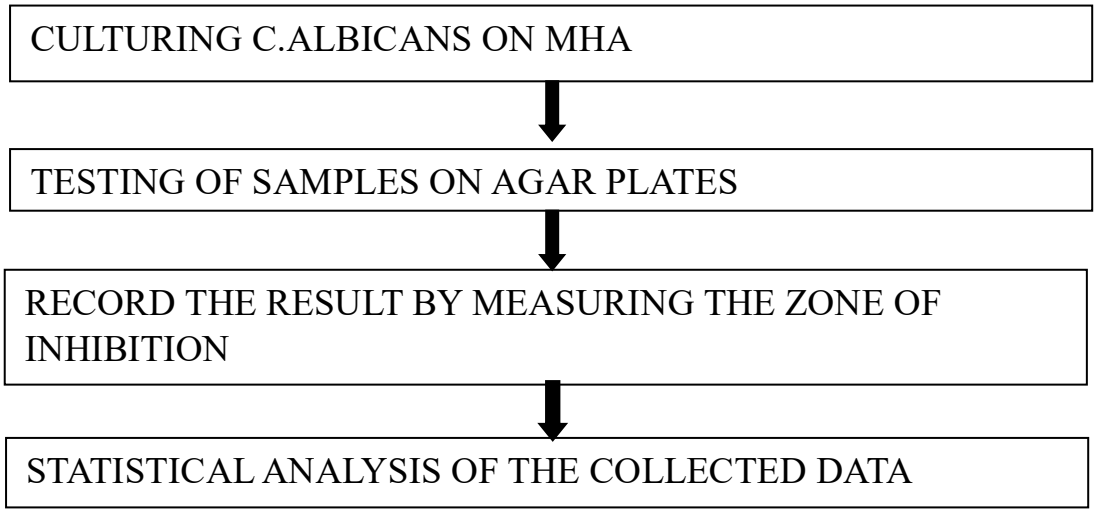
1. Circular Metallic Blocks (10 x 2) mm
2. Porcelain Jar
3. Stainless Steel Spatula
4. Flask
5. Clamp
6. Mechanical weighing scale
7. Mechanical Gram weights
8. Fractional Weights
9. Blood Agar plates
10. Mueller Hinton Agar (MHA)
11. Petri Dish
12. Sterile
13. Cotton swabs
14. Saline
15. Inoculating Loops
16. Test Tube
17. Bunsen burner
18. Spirit Lamp
19. Instrument holder
20. Test Tube holder
21. Test Tube stand
22. Sterile Carver

23. Sterile Excavator
24. Sterile forceps
25. Biosafety Cabinet
26. Incubator
27. Mixing Bowl
28. Straight spatula

STUDY PROTOCOL: -

STUDY DESIGN: -





Preparation of sample (DPI):

(i) 0.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.
(ii) 1.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.
(iii) 2.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.
(iv) 3.5% of Cassia fistula Extract incorporated into DPI acrylic resin material - 36 specimens each.

Methodology enumerated as:-

1.Fabrication of acrylic resin specimen

2. Sample Preparation

3.Testing of the samples

4. Statistical Analysis

Denture base material composed of heat-polymerized (DPI) acrylic resins is employed, and its handling adheres to the guidelines provided by the manufacturers. Thirty-six circular-shaped specimens (10 × 2mm) are crafted from each resin, (according to ADA Specification number 12) categorizing them

into four groups (n=3) based on the percentage of extract incorporated into the acrylic resin material.

The assessment encompasses the examination of both antibacterial and antifungal effects. The antimicrobial activity is gauged through the agar diffusion method against specific strains, including Gram-Positive Bacteria, such as Staphylococcus aureus, Gram-Negative Bacteria like Escherichia coli, and a fungal strain, Candida albicans.

Statistical analysis of the data is conducted using one-way ANOVA.

METHODOLOGY:-

i) Fabrication of metal blocks

A metal rod was taken of length 1.5m was used to fabricate the master circular metal blocks using laser. The input data for laser cutting was entered as 10mm diameter for each circular block with 2mm in depth/height respectively. A total of 12 blocks of 10mm diameter and 2mm height were fabricated out of 1.5m of metal rod.

ii) Weighing of extract per percentage

A sum total of 30g heat-cure acrylic (DPI) was calculated to fabricate 12 circular blocks of sample in consideration with the manufacturer's instructions. Hence, taking 30g as standard weight for all the percentages was calculated and removed from the acrylic resin (polymer) and the removed amount of polymer was replaced by weighed amount of extract according to the percentages respectively.

Total weight= 30g

For Control (C) only DPI acrylic resin material was taken to fabricate 36 samples.

Acrylic weight=30g
Extract weight= 0g

For 0.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.

0.5% of 30g= 0.15g
Acrylic weight=29.85g
Extract weight=0.15g

For 1.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.

1.5% of 30g= 0.45g
Acrylic weight=29.55g
Extract weight=0.45g

For 2.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.

2.5% of 30g= 0.75g
Acrylic weight=29.25g
Extract weight=0.75g

For 3.5% of Cassia fistula Extract incorporated into DPI acrylic resin material – 36 specimens each.

3.5% of 30g= 1.5g
Acrylic weight=28.95g
Extract weight=1.05g

iii) Mixing of extract+ heat cure acrylic material

The weighed amount of heat-cure acrylic material is mixed with the specific weighed amount of the Cassia Fistula Linn. extract as calculated in powder form. Modification of the material was performed by adding Cassia Fistula Linn. extract to the concentrations of 0.5%, 1.5% , 2.5% and 3.5% to the polymer powder, based on the total mass of the polymer-monomer mixture defined by the manufacturer's instructions as per for 12 blocks for heat -curing denture resin.

iv) Investment in the flask

Using plaster of paris, investment in made into the flask and the circular metal blocks are inserted into the POP while in the semisolid state. Thus, moulds are created for the curing of the heat-cure denture material.

v) Curing of extracts+ heat cure acrylic resin

After the powder and monomer were mixed, the heat-curing dough was placed in a disk-shaped molds 10 mm in diameter and 2 mm in height, invested into the plaster of paris. and put into standard denture flasks, pressed using a hydraulic press (Hydraulic dental press S-U-flask-press, Schuler-Dental GmbH & Co. KG, Ulm, Germany) (80 bar) and polymerized according to the manufacturer's instructions. In short, closed flasks were put for bench-curing for 24 hours and then were put in normal water, heated up to 100 °C/212 °F and let to boil for 90 min. After the polymerization process, heat-curing samples were finished using dental acrylic laboratory burrs without polishing.[48]

vi) Separating agar plates into Test (T) and Control (C)

On receiving the agar plates, preformulated for uniform thickness of agar in each petri dish. The agar plates were sourced from Biomereux with specific customisation in consideration with the procedure to be followed.

vii) Checking for 'Control (C)' and Test (T) Agar plates for contamination

One agar plate from each type was put in the incubator for 24 hours to check for any contamination and was marked as 'C' for control and antibiotic disc were put on another agar plate to check for the test 'T' of the agar plate. This was performed on both Mueller Hinton Agar plate and Blood Agar plate.

viii) Preparation of stock culture of E.coli, Staph. Aureus, C.albicans

The stock culture of E.coli, Staph. Aureus and C.albicans was prepared by private laboratory and was sourced from the same for investigation purposes.

ix) Culturing E.coli on MHA

Firstly the organism in the stock culture is to be test via gram staining and catalyse-coagulase kit. When viewed under the microscope, Gram-negative E. Coli will appear pink in color. In addition to this, E. coli has a positive catalase activity, The positive test is demonstrated by the immediate appearance of bubbles.
Then using the stock culture of E.coli, a cotton swab is taken is dipped into the stock culture of E.coli and is then swabbed over the entire petri plate for culturing E.coli on the Meuller Hinton Agar plate. The plates are rechecked for growth after 24 hours in the incubator.

x) Culturing S.aureus on Blood Agar

As we test the organism in the stock culture is to be test via gram staining and catalyse-coagulase kit. When viewed under the microscope, Staphylococcus aureus being a Gram- positive bacteria, stains purple by Gram stain. Staphylococcus (catalase positive) can be differentiated using the catalase test. The positive test is demonstrated by the immediate appearance of bubbles.

Then using the stock culture of S.aureus, a cotton swab is taken is dipped into the stock culture of S.aureus and is then swabbed over the entire petri plate for culturing S.aureus on the Blood agar plate. The plates are rechecked for growth after 24 hours in the incubator.

xi) Culturing C. albicans on MHA

Similarly C.albicans is also tested for the respective test and is then cultured on the petri plate of MHA via the swab culture method. The plates are rechecked for growth after 24 hours in the incubator.

xii) Testing of samples on agar plates

Once the culture is checked for proper growth over the agar plate, a sterile foreceps and sterile carver is taken and a well is dug using the instruments in order to place the specimen to be tested. The entire procedure is performed under the biosafety cabinet.

xiii) Record the result by measuring the Zone of Inhibition

The results are recorded by measuring the zone of inhibition after the specimens are set in the agar plates in the incubator. The measurement is done using a cm scale and the table is formulated based on the recordings made.

OBSERVATION AND RESULTS/

DATA MANAGEMENT AND STATISTICAL ANALYSIS

RESULTS:-

Data recorded for E.coli-

S.NO.	E.COLI.	0.5%	1.5%	2.5%	3.5%
1.		10	12	12	15
2.		10	10.4	12.4	16
3.		12	10	12	16.6
4.		12	12	13	18
5.		12	12.2	12.2	17
6.		10	12	13	18
7.		11	10	13	17.2
8.		10	10.4	12.6	18
9.		12	10.4	12.8	17.6
10.		10	12	12.6	17.2
11.		11	12.2	13	16.6
13.		12	12.2	13	18

Table - 1

Data recorded for S.aureus-

S.NO.	S. AUREUS	0.5%	1.5%	2.5%	3.5%
1.		10	11	13	14.6
2.		12	12.2	12.8	14.4
3.		10	10.8	12.8	14.6
4.		11.4	11	13.2	15
5.		12	11.6	12.4	14.8
6.		12.2	12	12.8	15
7.		10	10.8	13.2	14.6
8.		11	11	12.6	15.4
9.		12.2	11	13	15
10.		12	12.2	13.2	15
11.		10.6	10.6	12.6	15.2
13.		12	10.8	12	15.4

Table - 2

Data recorded for C..albicans-

S.NO.	C.ALBICANS	0.5%	1.5%	2.5%	3.5%
1.		12	12.4	14.6	18.4
2.		12.2	11.8	13	18
3.		12.2	12.8	14	18.8
4.		12	12.6	13.8	18.8
5.		12.4	12	13.6	18.4
6.		11.8	12.4	13.8	18
7.		11.8	12.6	13.6	18.8
8.		12	12.6	13.2	18
9.		12.4	12.8	14.4	18
10.		12.4	12.8	13.8	17.8
11.		11.8	12.8	13.2	18.8
13.		12	12.6	13.8	18

Table – 3

Statistical analysis of the collected data

The following statistical formulae were employed for the calculation of various parameters.

1. **Mean/Average**

   Mean/ Average is defined as the sum of all the given elements divided by the total number of elements.
   Mean= sum of all elements/ number of elements
   It is denoted by the letter **X**.

   $X=\sum X/n$
   Where n= No. of observations

2. Standard Deviation

The Standard Deviation of a statistical population ,a data set, or a probability distribution is the square root of its variance. Standard Deviation is widely used measure of the variability or dispersion,

It shows how much variation there is from the 'Average' or Mean. It is denoted by the letter **(σ)**

3. ANOVA Test

The Kruskal-Wallis Test is used to analyse the effects of more than two levels of just one factor on the experimental result. It is the non-parametric equivalent of the One-Way ANOVA.

The Friedman Test analyses the effect of two factors, and is the non-parametric equivalent of the Two-Way ANOVA.

The statistical tests used were ANOVA (Analysis of Varancc) test for Comparison of difference between mean values of more than 2 groups.

The fundamental technique is a partitioning of the total sum of squares (abbreviated SS) into components related to the effects used in the model.

The equation of ANOVA is given by:

SSTotal + SSError+ SSTreatements

So, the number of degree of freedom (abbreviated df) can be partitioned in a similar way:

dfTotal= dfErrort dfTreatements

4. Level of Significance (p- value)

It is the maximum probability of rejecting a correct null hypothesis.
In testing a given hypothesis, the maximum probability with which we would be willing to take risk is called Level of Significance of the Test. $p > 0.05$ - Non significant
$p < 0.05$- significant

p<0.01-Very Highly Significant

Descriptive statistics for various concentrations for each organism

Organism	Concentration	Mean	Std. Deviation	Minimum	Maximum
E. coli	0.50%	11.00	0.95	10	12
	1.50%	11.32	0.96	10	12.2
	2.50%	12.63	0.40	12	13
	3.50%	17.10	0.94	15	18
S. aureus	0.50%	11.28	0.92	10	12.2
	1.50%	11.25	0.59	10.6	12.2
	2.50%	12.80	0.36	12	13.2
	3.50%	14.92	0.32	14.4	15.4
C. albicans	0.50%	12.08	0.23	11.8	12.4
	1.50%	12.52	0.32	11.8	12.8
	2.50%	13.73	0.47	13	14.6
	3.50%	18.32	0.40	17.8	18.8

Table - 4

Comparison between concentrations for each organism

Organism	Concentration	Mean	Std. Deviation	F value	P value
E.coli	0.50%	11.00	0.95	132.501	**.000**
	1.50%	11.32	0.96		
	2.50%	12.63	0.40		
	3.50%	17.10	0.94		
S. aureus	0.50%	11.28	0.92	101.037	**.000**
	1.50%	11.25	0.59		
	2.50%	12.80	0.36		
	3.50%	14.92	0.32		
C. albicans	0.50%	12.08	0.23	730.454	**.000**
	1.50%	12.52	0.32		
	2.50%	13.73	0.47		
	3.50%	18.32	0.40		

Table- 5

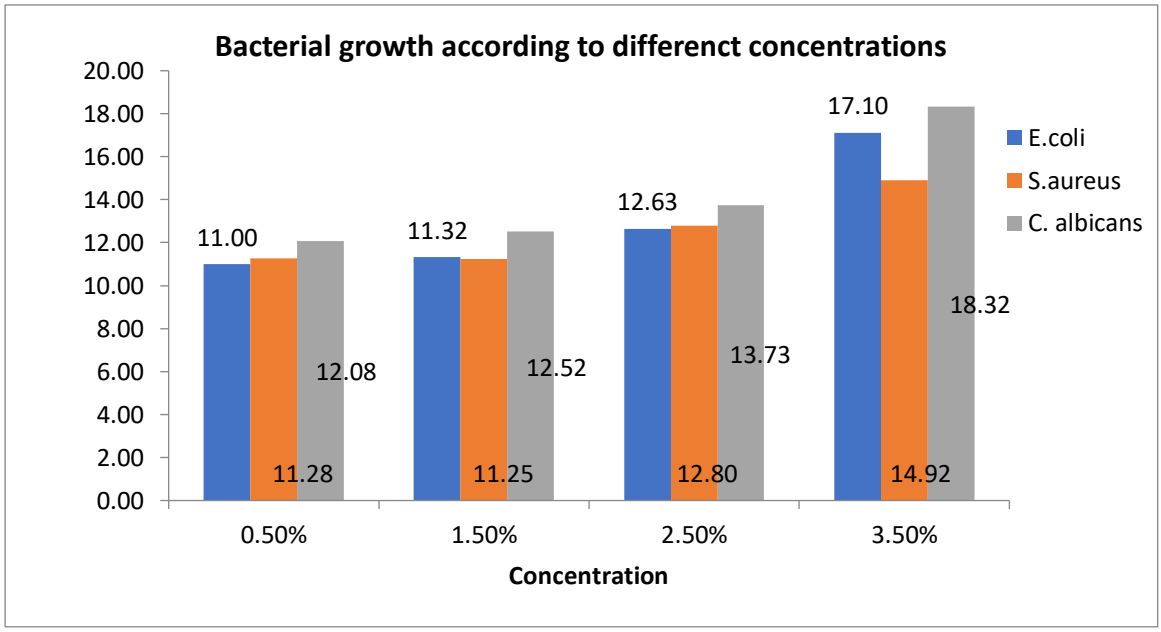
F and p value obtained from One way ANOVA. P value ≤ 0.05 is significant

Post hoc analysis

Organism	Group (I)	Group (II)	Mean Difference (I – II)	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
E.coli	1	2	-.32	.797	-1.24	0.61
		3	-1.63	**.000**	-2.56	-0.71
		4	-6.10	**.000**	-7.02	-5.18
	2	3	-1.32	**.002**	-2.24	-0.39
		4	-5.78	**.000**	-6.71	-4.86
	3	4	-4.47	**.000**	-5.39	-3.54
S. aureus	1	2	.03	.999	-.62	.68
		3	-1.52	**.000**	-2.17	-.87
		4	-3.63	**.000**	-4.28	-2.98
	2	3	-1.55	**.000**	-2.20	-.90
		4	-3.67	**.000**	-4.32	-3.02
	3	4	-2.12	**.000**	-2.77	-1.47
C.albicans	1	2	-0.43	**0.029**	-0.83	-0.03
		3	-1.65	**0.000**	-2.05	-1.25
		4	-6.23	**0.000**	-6.63	-5.83
	2	3	-1.22	**0.000**	-1.62	-0.82
		4	-5.80	**0.000**	-6.20	-5.40
	3	4	-4.58	**0.000**	-4.98	-4.18

Table -6

P value ≤ 0.05 is significant


Bacterial growth according to differenct concentrations
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
11.00
11.28
12.08
11.32
11.25
12.52
12.63
12.80
13.73
17.10
14.92
18.32
0.50%
1.50%
2.50%
3.50%
Concentration
E.coli
S.aureus
C. albicans

Graph-1

DISCUSSION

The integration of natural plant extracts into various materials has long been pursued as a means to leverage their therapeutic properties for diverse applications. Among the myriad of botanical candidates, Cassia fistula Linn., commonly known as the golden shower tree, has emerged as a promising source of bioactive compounds with potential medicinal benefits.[49-50] In recent years, there has been a growing interest in exploring the incorporation of Cassia fistula extracts into heat-cured acrylic resin, a widely used material in dentistry for fabricating dental prostheses such as dentures and crowns.[51]

Cassia fistula is renowned in traditional medicine for its diverse pharmacological activities, including antiulcer, antimicrobial, and antioxidant properties.[21-52-53] By integrating these bioactive constituents into acrylic resin formulations, researchers aim to enhance the therapeutic functionalities of dental prostheses while simultaneously addressing oral health concerns. The rationale behind this integration lies in the potential synergistic effects between the bioactive compounds present in Cassia fistula extracts and the properties of acrylic resin, such as its mechanical strength, biocompatibility, and durability.[54]

Furthermore, integrating Cassia fistula extracts into acrylic resin shows potential in tackling particular oral health issues, including the fight against oral pathogens, inflammation reduction, and tissue regeneration promotion. Additionally, by leveraging Cassia fistula's antimicrobial attributes, these altered acrylic resin compositions might aid in reducing the likelihood of oral infections and enhancing overall oral hygiene..[21 -52-53]

Research efforts in this area have aimed to elucidate the impact of Cassia fistula extract incorporation on the mechanical, chemical, and biological properties of acrylic resin-based dental materials.[56-57] Furthermore, studies have sought to optimize extraction and formulation techniques to maximize the retention and bioactivity of Cassia fistula compounds within the resin matrix.[58]

Overall, the incorporation of Cassia fistula Linn. extracts into heat-cured acrylic resin represents a promising avenue for developing novel dental materials with enhanced therapeutic functionalities. Through interdisciplinary collaborations between botanists, chemists, materials scientists, and dental professionals, this approach holds potential for advancing the field of biomaterials and improving oral healthcare outcomes.

The provided table presents insights into the efficacy of Cassia fistula Linn. extract at various concentrations against three distinct microorganisms: Escherichia coli, Staphylococcus aureus, and Candida albicans. Rather than focusing solely on numerical values, analyse the trends and implications of the

data. Firstly, it's noteworthy that as the concentration of Cassia fistula Linn. extract increases, there is a general trend of enhanced inhibition of microbial growth across all three microorganisms. This suggests a dose-dependent relationship between the concentration of the extract and its antimicrobial activity. Such a trend highlights the potential efficacy of Cassia fistula Linn. extract as a natural antimicrobial agent.

Moreover, the variation in inhibition patterns among the three microorganisms is noteworthy. For instance, Escherichia coli and Staphylococcus aureus exhibit similar responses to increasing concentrations of the extract, with a progressive increase in inhibition diameter. On the other hand, Candida albicans appears to demonstrate a more pronounced response, particularly at higher concentrations of the extract, indicating a potentially stronger susceptibility to the antimicrobial properties of Cassia fistula Linn.

Additionally, the range of inhibition diameters observed for each microorganism at different concentrations of the extract provides insights into the variability and robustness of the antimicrobial effects. A wider range may suggest greater variability in response to the extract, possibly influenced by factors such as microbial strain diversity or experimental conditions.

In conclusion, these findings suggest that Cassia fistula Linn. extract shows promise as a natural antimicrobial agent against Escherichia coli, Staphylococcus aureus, and Candida albicans. The observed dose-dependent inhibition of microbial growth emphasizes the potential versatility of Cassia fistula Linn. extract in various applications, including pharmaceuticals, food preservation, and beyond..

The provided table illustrates a comparative analysis of various concentrations of Cassia fistula Linn. extract across the tested microorganisms, namely Escherichia coli, Staphylococcus aureus, and Candida albicans. Each entry includes the mean inhibition diameter, standard deviation, F value, and p value derived from one-way ANOVA, serving to evaluate the significance of differences between concentrations.

Across the board, there's a discernible pattern of increasing mean inhibition diameter with escalating concentrations of the extract. This trend suggests a dose-dependent relationship, implying that higher concentrations yield greater inhibition of microbial growth. The standard deviation values offer insights into the variability within each concentration group, reflecting the dispersion of inhibition diameters around the mean.

The F value, along with the associated p value, obtained from the one-way ANOVA, provides statistical evidence regarding the significance of differences in inhibition diameters between concentrations. A significant F value, coupled with a p value below the threshold of 0.05, indicates a noteworthy variation in inhibition diameters among the concentrations tested.

Overall, the results underscore the potential efficacy of Cassia fistula Linn. extract as an antimicrobial agent against Escherichia coli, Staphylococcus aureus, and Candida albicans. The concentration-dependent inhibition of microbial growth suggests that higher concentrations of the extract may offer enhanced antimicrobial activity, thus highlighting its promising utility in various applications, including pharmaceuticals and food preservation.

In addition to that, Post hoc analysis is a statistical technique employed after conducting an initial analysis, such as ANOVA (Analysis of Variance) or another omnibus test, to delve deeper into significant differences between groups or treatments. It serves to identify specific pairwise differences that may exist among the groups or treatments that were compared. In the context of the provided data on Cassia fistula Linn. extract concentrations and microbial inhibition, post hoc analysis would enable researchers to determine which specific concentrations of the extract result in significantly different mean inhibition diameters for each microorganism tested, namely Escherichia coli, Staphylococcus aureus, and Candida albicans. There are various methods available for conducting post hoc analysis, each with its own assumptions and level of stringency.

The provided post hoc analysis table offers insights into specific pairwise differences in mean inhibition diameters between different concentration groups of Cassia fistula Linn. extract for each tested microorganism: Escherichia coli, Staphylococcus aureus, and Candida albicans. This analysis is crucial for identifying which concentrations exhibit statistically significant differences in microbial inhibition, beyond the omnibus ANOVA results.

For Escherichia coli, significant differences are observed between almost all concentration pairs, except for group 1 vs. group 2, where the mean difference is not statistically significant ($p = .797$). However, notably substantial differences are found between group 1 and group 3 (mean difference = -1.63, $p = .000$), as well as between group 1 and group 4 (mean difference = -6.10, $p = .000$). Similar significant differences are observed between other concentration pairs as well.

Likewise, for Staphylococcus aureus, significant differences are evident between most concentration pairs. Notably, the mean difference between group 1 and group 3 (mean difference = -1.52, $p = .000$) and between group 1 and group 4

(mean difference = -3.63, p = .000) are substantial and statistically significant. Significant differences are also observed between other concentration pairs.

For Candida albicans, significant differences are found between all concentration pairs. Particularly noteworthy differences are observed between group 1 and group 4 (mean difference = -6.23, p = .000) and between group 2 and group 4 (mean difference = -5.80, p = .000), indicating pronounced variations in microbial inhibition across different concentrations of the extract.

These findings highlight the importance of post hoc analysis in elucidating specific pairwise differences in mean inhibition diameters, thereby providing valuable insights into the effectiveness of different concentrations of Cassia fistula Linn. extract against Escherichia coli, Staphylococcus aureus, and Candida albicans. The significant differences identified underscore the potential utility of specific concentrations of the extract as effective antimicrobial agents against these microorganisms.

The review of literature on Studies have demonstrated the extract's efficacy in mitigating gastric ulcers, inhibiting bacterial and fungal growth, and even acting as an acaricidal agent against ticks. These findings suggest a broad spectrum of therapeutic potential for Cassia fistula Linn. extract across different biological systems.[21]

Correlating these findings with the descriptive statistics for various concentrations of the extract against different organisms provides valuable insights into its dose-dependent effects on microbial inhibition. For instance, the literature highlights the extract's potent antibacterial activity against Gram-positive bacteria, corroborated by the significant inhibitory effects observed in the descriptive statistics. The presence of phytochemicals such as alkaloids, flavonoids, and saponins, as reported in the literature, likely contributes to its antimicrobial efficacy, as evidenced by the observed zone of inhibition in agar disc diffusion tests.Similarly, the literature underscores the extract's antifungal potential, particularly against Candida species, which aligns with the descriptive statistics demonstrating significant inhibition of Candida albicans growth at higher concentrations of the extract.[21] The presence of phenolic compounds, including rhein, in the extract as reported in the literature, likely plays a crucial role in its anticandidal activity, as indicated by the observed correlations between minimum inhibitory concentrations, cytotoxicity profiles, and ergosterol inhibition.[24]

Furthermore, the literature highlights the extract's acaricidal properties, supported by descriptive statistics showing concentration-dependent effects on tick

mortality and reproductive processes. The presence of bioactive compounds in the extract, such as those identified in the phytochemical analysis, likely contributes to its acaricidal efficacy, leading to complete inhibition of egg hatching at higher concentrations.[21]

N R Bhalodia and V J Shukla (2011) expanded on the The antimicrobial properties of Cassia fistula Linn. leaves, particularly their antibacterial and antifungal activities, were examined. The results confirmed the inhibitory effects against clinically relevant bacterial and fungal strains, with hydroalcohol extracts demonstrating significant antimicrobial effectiveness. The study underscored the potential of the extract as a substitute for traditional antibiotics, underscoring its importance in therapeutic applications.[23]

Furthermore, Irshad, Sheikh Shreaz, et al. (2011) elucidated The antifungal properties of Cassia fistula Linn., highlighting its potential as an innovative antifungal agent, were explored. Their research unveiled substantial antifungal effectiveness against Candida strains, linked to the existence of phenolic compounds and rhein.. The study emphasized the importance of phytochemical screening in identifying bioactive constituents and exploring their therapeutic applications in combating candidiasis.[24]

N. L. Martins Almeida and Luiz Leonardo Saldanha et al. (2017) investigated the antimicrobial effects of Equisetum giganteum and Punica granatum fractions added to denture adhesive against C. albicans biofilm. Their study demonstrated enhanced anti-biofilm activity of the adhesive with herb extract mixtures, suggesting a potential application in Denture Stomatitis treatment and prevention.[28]

The literature review encompasses various studies exploring the therapeutic potential of natural extracts and their effects on microbial activity, particularly on organisms relevant to oral health and infection control. These investigations shed light on the efficacy of incorporating herbal extracts into dental materials and their impact on microbial biofilms. For instance, studies by Antunes and Carolina (2014) and Al-Nema and Al-Irhayim (2014) highlighted the antimicrobial efficacy of green tea extract and medicinal plant oils against Candida albicans and other prevalent microorganisms in the oral cavity was assessed These findings highlight the potential of natural compounds in addressing oral infections and preventing biofilm formation..[26-27]

Furthermore, McCormack, Smith et al. (2015) offered insights into the prevalence of Staphylococcus aureus in the oral cavity, emphasizing the necessity for efficient infection control strategies. The occurrence of S. aureus in the oral

microbiome underscores its potential as a source of cross-infection and underscores the significance of comprehending its dynamics in managing oral health.[16]

Besides, Martins Almeida and Saldanha et al. (2017) investigated the antimicrobial effects of herbal fractions added to denture adhesive against Candida albicans biofilm. Their study demonstrated enhanced anti-biofilm activity with herb extract mixtures, suggesting a potential adjunctive therapy for Denture Stomatitis treatment and prevention.[28]

The investigation by Z.S. Mawlood and A.H. Naji (2021) explored how Bergamot Essential Oil (BEO) affects the mechanical properties of heat-cured acrylic denture bases, which are crucial for their strength and ability to resist fractures. Ninety samples of heat-cured acrylic resin were divided into groups with varying concentrations of BEO (0%, 5%, and 6% by volume). Tests were conducted to assess transverse strength, impact strength, and surface roughness. Statistical analyses, including One-Way ANOVA, Tukey HSD, and Dunnett T3 tests, were employed to analyze the results. The findings revealed a significant increase in transverse strength with the addition of 5% and 6% BEO, while impact strength remained consistent across all concentrations.

Samples containing 5% and 6% BEO exhibited a significant reduction in surface roughness compared to the control group. These findings indicate that incorporating Bergamot Essential Oil could potentially enhance the mechanical characteristics of heat-cured acrylic denture bases, specifically by improving transverse strength and promoting smoother surfaces, all while maintaining impact strength.[35]

In conjunction with these findings, the descriptive statistics for various concentrations of herbal extracts, as presented in the results section, provide quantitative data on their antimicrobial efficacy. By correlating the literature review with the statistical analysis, a comprehensive understanding of the potential therapeutic benefits of natural extracts in dental applications emerges. These findings not only underscore the importance of exploring alternative therapies for oral infections but also provide valuable insights into the development of novel dental materials with enhanced antimicrobial properties.

CONCLUSION

Based on the results of the one-way ANOVA and post hoc analysis, significant differences in concentrations were observed among the tested organisms (E. coli, S. aureus, and C. albicans) at various concentration levels (0.50%, 1.50%, 2.50%, and 3.50%). The mean concentrations varied significantly across different organisms and concentration levels, as indicated by the F-values and associated p-values.

For E. coli, significant differences were found between all concentration groups (0.50%, 1.50%, 2.50%, and 3.50%) except between the 0.50% and 1.50% concentrations.

Similarly, for S. aureus, significant differences were observed between all concentration groups except between the 0.50% and 1.50% concentrations.

For C. albicans, significant differences were found between all concentration groups, indicating a distinct response to different concentrations of the tested substance.

These findings suggest that the efficacy of the substance varies significantly depending on the organism and concentration level, highlighting the importance of tailoring concentrations for effective antimicrobial activity against specific pathogens.

SUMMARY

The utilization of natural plant extracts in various materials has been a longstanding pursuit to harness their therapeutic potential for a range of applications. Among these botanical options, Cassia fistula Linn., commonly known as the golden shower tree, has emerged as a promising source of bioactive compounds with potential medicinal advantages. In recent times, there has been increasing interest in investigating the inclusion of Cassia fistula extracts into heat-cured acrylic resin, a prevalent material in dentistry used for crafting dental prostheses like dentures and crowns. Cassia fistula is well-regarded in traditional medicine for its diverse pharmacological benefits, including antiulcer, antimicrobial, and antioxidant properties.

By integrating these bioactive components into acrylic resin formulations, researchers seek to augment the therapeutic attributes of dental prostheses while simultaneously tackling oral health issues. The rationale behind this integration lies in the potential synergies between the bioactive compounds found in Cassia fistula extracts and the inherent properties of acrylic resin, such as mechanical strength, biocompatibility, and longevity.

The study protocol involved the preparation of samples, specifically DPI (Denture Base Material) incorporating different percentages of Cassia fistula Extract. This included the integration of 0.5%, 1.5%, 2.5%, and 3.5% of Cassia fistula Extract into DPI acrylic resin material, with each concentration yielding 36 specimens. In total, there were 144 specimens divided into four groups based on the varying concentrations of Cassia fistula Extract. This systematic approach facilitated the evaluation of the effects of different extract concentrations on the properties of DPI acrylic resin material, providing valuable insights into its potential therapeutic benefits for dental prostheses.

The methodology involves: 1. Fabrication of circular acrylic resin specimens following manufacturer guidelines. 2. Preparation of 36 specimens per group, categorized by the percentage of Cassia fistula Extract incorporated. 3. Testing includes assessment of antibacterial and antifungal effects using agar diffusion method against Staphylococcus aureus, Escherichia coli, and Candida albicans. 4. Statistical analysis is performed using one-way ANOVA to analyze the data obtained from the tests.

The meticulous methodology implemented in this study ensures precision and reproducibility in sample preparation. Through laser cutting, the fabrication of metal blocks guarantees uniformity, with each block measuring 10mm in diameter and 2mm in height. The careful adjustment of heat-cure acrylic resin, coupled with the incorporation of Cassia Fistula Linn. extract at varying percentages, reflects the deliberate design of the experiment. By meticulously

weighing and mixing the extract with the resin powder, thorough dispersion is achieved, optimizing the interaction between the extract and the resin. Furthermore, the use of plaster of Paris for flask investment creates molds that accurately accommodate the samples, facilitating the curing process. This methodical approach underpins the reliability of the study's findings regarding the impact of Cassia Fistula Linn. extract on DPI acrylic resin material.

The methodology further encompasses several steps to ensure the accuracy and reliability of the study's findings. It is then followed by the curing process which involves mixing the powder and monomer of heat-curing acrylic resin, followed by placing the resulting dough into disk-shaped molds. These molds, measuring 10mm in diameter and 2mm in height, are then invested into plaster of Paris and placed into standard denture flasks. These flasks undergo pressing using a hydraulic press and are polymerized according to the manufacturer's instructions. Following bench-curing for 24 hours and boiling in water, the samples are finished without polishing.

Agar plates are then divided into Test and Control groups, and each group is checked for contamination. Meanwhile, stock cultures of E.coli, Staph. Aureus, and C.albicans are prepared and cultured on appropriate agar plates. The growth of these cultures is examined after incubation to ensure viability.

Once the cultures are deemed suitable, samples are tested on agar plates. A sterile foreceps and carver are used to dig a well in the agar plates, and the specimens are placed inside. The entire process is conducted within a biosafety cabinet to maintain sterility.

After incubation, the results are recorded by measuring the zone of inhibition using a centimeter scale. This data is then analyzed to draw conclusions regarding the effectiveness of the tested specimens against the target microorganisms.

Based on the outcomes of the one-way ANOVA and subsequent post hoc analysis, notable disparities in concentrations were noted among the assessed organisms (E. coli, S. aureus, and C. albicans) at different concentration levels (0.50%, 1.50%, 2.50%, and 3.50%). The mean concentrations exhibited significant variations across diverse organisms and concentration levels, as evidenced by the F-values and corresponding p-values.

For E. coli, noteworthy differences were discerned among all concentration groups (0.50%, 1.50%, 2.50%, and 3.50%), except for the 0.50% and 1.50% concentrations. Similarly, in the case of S. aureus, substantial disparities were observed among all concentration groups, barring the 0.50% and 1.50% concentrations.

Regarding C. albicans, significant differences were identified among all concentration groups, signifying a distinct reaction to varying concentrations of the evaluated substance. These findings underscore the variability in the substance's efficacy based on the organism and concentration level, underscoring the necessity of tailoring concentrations to achieve effective antimicrobial activity against specific pathogens.

Summarizing in the entirety, the study's findings shed light on the intricate relationship between concentration levels of the tested substance and its efficacy against various pathogens. The observed significant variations in effectiveness across different organisms underscore the need for a tailored approach in determining optimal concentrations for combating specific microbial threats. This emphasizes the importance of personalized concentration adjustments to achieve maximum antimicrobial activity while minimizing the risk of resistance development. Such tailored strategies hold promise in enhancing the overall effectiveness of antimicrobial interventions, thereby contributing to more targeted and efficient management of infectious diseases in clinical and public health settings.

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Date: 02.11.2023

To,
Indian Council of Medical Research
V. Ramalingaswami Bhawan
P.O. Box No. 4911. Ansari Nagar
New Delhi - 110029

Subject- Change in guide for ICMR-STS 2023

Respected Sir/ Ma'am,

With due respect this is to state that Miss. Riya Rupam (STS ID- 2023-07744), student of Seema Dental College and Hospital, B.D.S IV year, Roll No.-2053, would humbly like to request a modification in the academic guidance for ICMR STS 2023 research project. Due to the resignation of former guide Dr. Himanshu Aeran (Former principal, HOD and Professor of Department of Prosthodontics, Crowns and Bridges, Seema Dental College and Hospital) a change in the academic guidance is needed. Dr. Varun Kumar (New HOD of Department of Prosthodontics, Crowns and Bridges, Seema Dental College and Hospital) has consented to assume the role of guide of Miss. Riya Rupam in her forgoing ICMR-STS 2023 project.

Thereby, requesting the authorities at ICMR, to kindly allow resuming the project with the changes as mentioned above.

Thanking you,

Seema Dental College & Hospital Rishikesh
PRINCIPAL

Dr. P. Narayana Prasad
Principal
Seema Dental College and Hospital
Rishikesh

TITLE:- To Evaluate and Compare the Antibacterial and Antifungal Properties of Cassia fistula Extracts at different percentages Incorporated into Acrylic Resin

INTRODUCTION-

Our oral cavity harbors the microflora which is inclusive of thousands of different microorganisms essential to protect against infectious agents that can attack the human body. However, on the other hand certain conditions including immunosuppression, malnutrition, poor oral hygiene, misuse of antibiotics, trauma and the misuse of removable prosthesis, may increase the risk of developing oral infections, which is why the recent advancements alongside naturopathy has led to the introduction of various plant-based extracts that subsequently leads to the increment in the anti-bacterial and anti-fungal properties.

This study is supposed to be caried out with an objective to investigate the antibacterial and antifungal potentials of leaves of Cassia fistula Linn. In the present study, the microbial activity of hydroalcohol extracts of leaves of Cassia fistula Linn. (an ethnomedicinal plant) was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains.

The antibacterial and antifungal activities of extracts (5, 25, 50, 100, 250 µg/ml) of Cassia fistula are to be tested against two Gram-positive—Staphylococcus aureus, Streptococcus pyogenes; two Gram-negative—Escherichia coli, Pseudomonas aeruginosa human pathogenic bacteria; and three fungal strains— Candida albicans , Aspergillus niger, Aspergillus clavatus.

The phytochemical analyses of the plants were carried out. The microbial activity of the Cassia fistula was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

OBJECTIVE:- The purpose of this in-vitro study is to compare and evaluate the antimicrobial activity of acrylic resins containing different percentages of Cassia fistula Linn. extracts into acrylic resins from two different companies.

The purpose of this in-vitro study is to compare and evaluate the antifungal activity of acrylic resins containing different percentages of Cassia fistula Linn. extracts into acrylic resins from two different companies.

This study would also bring about the noticeable alterations into the mechanical properties due to the incorporation of the Cassia fistula Linn. extracts.

METHODOLOGY:-

Study Protocol :-

Preparation of sample A (Pyrax):

A-(i) 0.5% of Cassia fistula Extract incorporated into Pyrax acrylic resin material - 5 specimens each.

A- (ii) 1.5% of Cassia fistula Extract incorporated into Pyrax acrylic resin material - 5 specimens each.

A-(iii) 2.5% of Cassia fistula Extract incorporated into Pyrax acrylic resin material - 5 specimens each.

A-(iv) 3.5% of Cassia fistula Extract incorporated into Pyrax acrylic resin material - 5 specimens each.

Preparation of sample B (DPI):

B-(i) 0.5% of Cassia fistula Extract incorporated into DPI acrylic resin material - 5 specimens each.
B-(ii) 1.5% of Cassia fistula Extract incorporated into DPI acrylic resin material - 5 specimens each.
B-(iii) 2.5% of Cassia fistula Extract incorporated into DPI acrylic resin material - 5 specimens each.
B-(iv) 3.5% of Cassia fistula Extract incorporated into DPI acrylic resin material - 5 specimens each.

Methodology that will be used in the study:-

1.Fabrication of acrylic resin specimen

2. Sample Preparation

3.Testing of the samples

4. Statistical Analysis

Two heat-polymerised (PYRAX and DPI) acrylic resins are to be used. The materials were handled according to the manufacturers' instructions. Forty rectangular-shaped specimens (8 × 10 × 4mm) will be fabricated from each resin and assigned to 8 groups (n=5) according to their percentage of extract incorporated into the acrylic resin material.

Anti-Bacterial and Anti-fungal effects are to be evaluated. The antimicrobial activity against two strains of Gram-Positive Bacteria namely- Staphylococcus aureus, Streptococcus pyogenes and two gram negative- Escherichia coli, Pseudomonas aeruginosa human pathogenic bacteria is to be assessed by agar diffusion method. Data were analysed statistically by one-way ANOVA.

Implication- Since the study is entirely going to be about the investigation post the incorporation of the antibacterial and antifungal properties via the introduction of Leaves extract of Cassia Fistula. The study would bring about the extent of possible incorporation of the extract according to the percentage while it does not hamper the mechanical strength and bring about the best possible advancement in the use of acrylic resin.
The antibacterial and antifungal activities of extracts (5, 25, 50, 100, 250 µg/ml) of Cassia fistula that are to be tested against two Gram-positive—Staphylococcus aureus, Streptococcus pyogenes; two Gram-negative—Escherichia coli, Pseudomonas aeruginosa human pathogenic bacteria; and three fungal strains— Candida albicans , Aspergillus niger, Aspergillus clavatus, via the agar diffusion method should bring about the results in favour that may serve as the development of new pharmaceuticals research activities.

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HRD/Head/STS-2023/01
Date: 14.08.2023

SHORT-TERM STUDENTSHIP (STS-2023)

Proposal Result

1. The final list of students selected for STS-2023 program is displayed below in order of their STS-2023 Reference ID.
   Please note: Always quote your STS Reference ID for any future correspondence emails to ICMR.
2. The 'Selected' students as listed in table below for STS-2023 may carry out the proposed research work and prepare the report in any two months *w.e.f.* 17th August, 2023 to 29th December, 2023
3. Research should be done after appropriate Institutional Ethics Committee (IEC) approval has been obtained. The ethical clearance letter has to be submitted along with report (IEC approvals should be taken latest by 30th November, 2023. Any IEC letter/approvals taken after 30th November, 2023 will not be considered as the student will not get two months of time for carrying out the work by the deadline given above).
4. The online report submission dates will be declared by the ICMR in due course of time.
5. The proposal result declared below by the ICMR is final. No further correspondence in the matter will be entertained.

Kindly read the detailed report preparation guidelines and instructions given on the ICMR website

GENERAL ENQUIRIES to be sent through email to stshrd2017@gmail.com or call on extn.no. 306, 369

Final result list of STS–2023 approved proposals in order of reference ID.

S. No.	STS Reference ID
765.	2023-07744