COMPARISON & EVALUATION OF ANTIMICROBIAL EFFICACY OF FOUR DIFFERENT PLANT EXTRACTS: AN IN VITRO STUDY

Anant Singh[1*] Neeraj Kant Panwar[2], Abhay Agarwal[3], Alaka Vaish[4], Asib Ahmed[5], Tanya Agarwal[6]

Abstract

Aim: To compare and evaluate the anti-cariogenic properties of the different plant extracts against various cariogenic micro-organism.

Materials and Methods: In phase 1 the anticariogenic efficacy of 4 different herbal extracts namely *ocimum sactum* (Tulsi), *Curcuma longa* (Turmeric), *Syzygium aromaticum* (Laung) and *Tinospora cordifolia* (Giloy), will be evaluated against two strains of bacteria viz. S.mutans and L.acidophilus. In the second phase of the study, decay depth in tooth samples of 10 in each group will be compared within 4 groups using polarised light microscope.

Results: The intragroup comparison for the different time intervals was done using Repeated Measures ANOVA to find the difference between the individual time intervals The level of the significance for the present study was fixed at 5%

Conclusion: the anti-cariogenic efficacy of "Laung" (*Syzygium acromaticum*) was highest followed by "Tulsi" (*Ocimum sanctum*) than "Haldi" (*Curcuma Longa*) and "Giloy". (*Tinospora cordifolia*) Giloy was found to be least effective among these against S. mutans and L. Acidophilus micro-organisms. The caries extension in dental tissue treated with different herbal extracts with aid of Stereo-light Microscope showed Giloy with higher caries incidence.

[1*,2,3,4,5,6]Kalka Dental College, Meerut

***Corresponding Author:** Anant Singh
*Kalka Dental College, Meerut

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INTRODUCTION

Dental caries is the most widespread chronic disease worldwide despite of the most advancement treatment modalities available today. In baby teeth it affects about 620 million people or 9% of the population. In the United States, dental caries is the most common chronic childhood disease, being at least five times more common than asthma.[1] Dental caries is a chronic infectious disease that shows multifaceted aetiology and leads to the destruction of dental hard tissues. There are different kinds of bacteria involved in tooth decaying process. Such as mutans group of streptococci, *S. salvarious, S. mitis, S. milleri, S. oralis, S. sanguis, Lactobacillus casei, Actinomyces viscosus*.[2]

Fermentation of sugar presents in a diet by oral bacteria results in a decrease in the pH of oral cavity leading to accumulation of plaque and demineralization of enamel and finally formation of dental caries. The acid produced as the substrate by the microbe will cause the dissolution of the surface enamel and exposes the underlying avascular mineralized connective tissue matrix of dentine, which is prone to invasion. This occurs by migration of bacteria into the network of tubules occupied by processes of the pulpal odontoblasts.[3]

Streptococcus mutans (S.mutans) is considered to be the chief caries causing bacteria in humans. The early stage of caries invasion involves lactobacilli, Actinomyces spp., veillonellae, and mutans streptococci. ***Lactobacilli acidophilus*** have been reported to have high occurrence in both superficial and deep caries, though they are not suspected of being involved in bacterial invasion of nonexposed dental pulp.[1]

Among the vast treasure of herbs available, *Tinospora cordifolia* (Giloy), *Syzygium aromaticum* (Laung), *Ocimum sanctum* (Tulsi), and *Curcuma longa* (Haldi) are herbs which were commonly used for prevention and treatment of various diseases from ancient times.[4]

For its numerous therapeutic benefits, *Ocimum sanctum* L. (also known as Ocimum tenuiflorum, or Tulsi) has been utilised in Ayurveda for thousands of years because of diverse healing properties. There is a dearth of research on the antibacterial activity of Ocimum sanctum L against caries causing organisms like Streptococcus mutans and Lactobacillus acidophilus.[5] *Tinospora cordifolia*, also known as "Guduchi" in Sanskrit and “Giloy” in hindi. Due to its alleged medicinal properties, including anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, and anti-neoplastic activities, the plant has recently attracted the attention of researchers from all over the world. [6]

Syzigium aromaticum L., sometimes known as laung, A common use of S. aromaticum buds' essential oil is in medicine, particularly in the field of dentistry. The essential oil is efficient against a variety of bacteria, including *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Salmonella enteritidis*, *Campylobacter jejuni* and *Staphylococcus aureus*.[7] Curcuma longa L. (C. longa), popularly known as turmeric, It has also been used in Oriental folk medicines to treat infectious diseases (e.g. sinusitis, cough), cholecystitis and cholangitis and used as a therapy for hepatic disorders, rheumatism and anorexia (Kim, 1989).[8]

In the current study, antimicrobial efficacy and anti-cariogenicity of the plants extracts will be checked against the strains of the streptococcus mutans and lactobacillus acidophilus to find out their efficacy against strains.

AIM & OBJECTIVE

AIM: To evaluate and compare the anti-cariogenic properties of the different plant extracts against various cariogenic micro-organisms.

OBJECTIVE:

- To Evaluate and compare the anti-cariogenic efficacy of different herbal extracts with respect to different micro-organisms
- To categorize anti-cariogenic potency of herbal extracts
- To check the caries extension in dental tissue treated with different herbal extracts with aid of Stereo light Microscope

MATERIAL & METHOD

The present study was carried out in the Department of Pedodontics and Preventive Dentistry, Kalka dental college and Hospital, Meerut in association with Helix Microbiology laboratory, Noida to investigate the anti-cariogenic properties of the different plant extracts against various cariogenic micro-organisms.

The herbal extracts that were used in the study are Giloy (*Tinospora cordifolia)*, Laung (*Syzygium aromaticum*), Haldi (*Curcuma longa*), Tulsi (*Ocimum sanctum*) obtained from Bhagvati Herbal and Healthcare Private Limited. (Ahmedabad).

The bacterial strains of *Streptococcus mutans* and *Lactobacillus acidophilus* used in the study were ordered from "Microbial Type Culture Collection and Gene Bank – Council of Scientific Industrial Research, Chandigarh".

The total of 40 teeth taken as samples were collected from the, Department of Oral and maxillofacial Surgery, Kalka Dental College, Meerut and were processed at Helix Laboratory, Noida and teeth were evaluated at Department of Microbiology, Subharti Medical College, Meerut.

Inclusion Criteria for teeth

- Non -Carious Teeth
- Teeth indicated for extraction due to periodontally compromised condition
- Teeth indicated for extraction for orthodontic treatment purpose.

Exclusion Criteria for teeth

- Carious teeth

MATERIALS USED

I. TYCSB agar base (Himedia, Mumbai, Maharashtra) (Fig. 3)
II. Lactobacillus MRS Broth (Titan Biotech Ltd, Bhiwadi)(Fig. 2)
III. Agar Agar Type 1 (Central Drug House, New Delhi) (Fig. 2)
IV. Mueller-Hindon Agar (Titan Biotech Ltd, Bhiwadi) (Fig. 2)
V. Herbal extracts (Bhagvati Herbal and Healthcare Pvt. Ltd., Ahmedabad) (Fig 1)
a. -Giloy (*Tinospora cordifolia)* extract
b. -Laung (*Syzygium aromaticum*) extract
c. -Haldi (*Curcuma longa*) extract
d. -Tulsi (*Ocimum sanctum*) extract
VI. Bacterial culture –
a. Streptococcus Mutans (CSIR, Chandigarh) (Fig. 4)
b. Lactobacillus Acidophilus (CSIR, Chandigarh)
VII. Cotton
VIII. 10% Formalin (Swapnika, Hisar, Haryana)
IX. Rubber cups (Chromadent Prophy, Mumbai)
X. Saline (0.45% w/v) (Primeera Healthcare, Hyderabad)
XI. Nail Polish- Blue, Green, Red and Yellow (Elle's 18 and Coloressence) (Fig. 6)
XII. Sterilisation pouch (Oro) (Fig. 5)
XIII. Test Tubes 5ml (Borosil) (Fig. 5)
XIV. Petridish (Borosil)
XV. Pumice Powder (Neelkanth, Jodhpur, Rajasthan)
XVI. Sticky Wax
XVII. Nitric acid (Qualigens fine chemicals, Mumbai) (Fig. 7)

ARMAMENTARIUM

- Incubator (Jindal, New Delhi)
- B-Class Autoclave (Sun, New Delhi)
- Stereo-Microscope (Olympus, Japan)

METHODOLOGY

The study was conducted in two phases:

In phase I, the anticariogenic efficacy of four different herbal extracts, namely *Ocimum Sanctum* (Tulsi), *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), and *Tinospora cordifolia* (Giloy), were evaluated against two strains of bacteria viz. S. mutans and L. Acidophilus known to be common etiological agents of dental caries, at different time interval using the agar diffusion test (ADT).

The bacterial strains were cultured in microbiology lab of the CSIR- Central Scientific Research organization, Chandigarh. The specific culture medium was used to grow the specific bacteria. This ensure that no contamination is present while the bacterial culture is prepared.

Preparation of Culture Medium

For the study, three culture medium were selected. The DeMan, Rogosa and Sharpe broth (MRS) were used to make the cultural medium for the culture of *Lactobacillus acidophilus*. The TYCSB agar base was used for the culture of the *Streptococcus mutans* and Mueller Hinton agar is used to maintaining the colonies of the both the strains that is *Streptococcus mutans* and *Lactobacillus acidophilus*.

Preparation of MRS broth

For the preparation of MRS broth agar, 5.5g of MRS broth was taken and measured at weight machine. (Fig. 8) The laboratory grade distilled water was used to dilute the broth. The broth is diluted with 100ml of distilled water. To solidify the solution additionally 2g of agar agar type 1 is added in the medium. The solution was mixed well in the flask and later on poured in the jar for autoclaving (Fig. 9) The solution was placed in the B-class autoclave and was autoclaved at 121 degrees for 15min at 15psi. (Fig. 10) After autoclaving the solution was poured in the petridish and allowed to set for 2 hours. (Fig. 11&12)

Preparation of TYCSB Agar Base

For the preparation of Tryptone Yeast Extract Cystine w/Sucrose and w/o Bacitracin Agar base, 24.9g of TYCSB agar base was taken and measured at weighing machine. The laboratory grade distilled water was used to dilute the agar. The TYCSB base was diluted with 100ml of distilled water. The prepared solution was mixed well in the flask and

later on poured in the jar for autoclaving. The solution was placed in the B-class autoclave and autoclaved at 121 degrees for 15min at 15psi. After autoclaving the solution was poured in the petridish and allowed to set for 2 hours.

Preparation of MH agar
For the preparation of Mueller-Hindon (MH) agar, 13.5g of nutrient agar was taken and measured at weight machine. The laboratory grade distilled water was used to dilute the agar. The MH agar was diluted with 100ml of distilled water. The prepared solution was mixed well in the flask and later on poured in the jar for autoclaving. The solution was placed in the B-class autoclave and autoclaved at 121 degrees for 15min at 15psi. After autoclaving the solution was poured in the petridish and allowed to set for 2 hours

Preparation of Bacteria
The bacterial strains taken from the MTCC were bought in the powdered form. According to the given instruction, the 0.4 ml of distilled grade water was added in the bacterial strains to make bacteria active. (Fig.13) The *Streptococcus mutans* strains were taken in the sterile loop. The loop containing the colonies were applied in the streak over the blood agar and placed in the incubator for 48 hours. Similarly, the *Lactobacillus acidophilus* solution was applied in the streaks over the MRS agar plate and placed in the incubator for 48 hours.

Confirmation of Bacteria
Both the plates after the culture were checked with the gram staining to re confirm the bacteria that grows over the culture. (Fig. 14,15 &16)

Transferring of Bacteria
The 4-5 colonies of *Streptococcus mutans* were taken with the sterile loop. The bacteria were diluted in 5ml of saline water. It produced the turbidity of 1 on Macfarland scale. The sterilised cotton were dipped in the bacterial solution and gently applied over MH agar plate to ensure the proper distribution of bacteria over the agar plate. Similarly with the *Lactobacillus acidophilus,* 4-5 colonies were taken through sterile loop. The colonies were diluted in 5ml of saline water. The turbidy checked on Macfarland scale was 1. The sterilised cotton is dipped in *Lactobacillus acidophilus* solution and applied over the MH agar plate.

The total of 8 plates were prepared. 4 plates cultures with *Streptococcus mutans* and remaining for cultured with *Lactobacillus Acidophilus*. All the plates were kept in incubator for 48 hours. (Fig. 22)

Herbal Extract
After 2 hours, the plates were taken and 5 punches of 10mm were done over the MH agar. (Fig. 21) All the four herbal extracts were poured in the each in punched agar. One controlled punch is filled with saline water.

All the petridish were placed in the incubator, diameter of the zone of inhibition were measured at the interval of 24, 48, and 72 hours

In phase II, of the study, the overall decay depth in tooth sample were compared after treatment with different herbal extracts, i.e., *Ocimum Sanctum* (Tulsi), *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), and *Tinospora cordifolia* (Giloy) by using Sterio-Microscope.

Preparation of Samples.
Just after the extraction, the teeth were placed in 0.9% normal saline solution, and then kept in 10% solution of formalin for disinfection. After a week, with the help of periodontal curettes, the remaining soft dental tissue were removed and cleaning was performed using fluoride-free pumice and rubber cups. Teeth surfaces were covered using nail polish except a window of 5 × 5 mm which is on the buccal surface.

Colour Coding of Samples
All the 40 teeth, were divided into 4 groups, 10 teeth in each sample. The 4 groups are Tulsi, Giloy, Haldi, Laung. The Tulsi teeth were painted with red colour, the Giloy samples with yellow, the Haldi samples with green and Laung with blue. (Fig. 23)

Disinfection of sample
For sterilization, teeth were put in sterilisation pouch and kept in an autoclave at 121°C and a pressure of 15 pounds for 15 minutes. After that, teeth apices were sealed using sticky wax.

Preparation of Artificial Cariogenic Medium
Nutrient agar broth was used which was sterilized in an autoclave. The artificial cariogenic medium solution was prepared using saline, nutrient broth and extracted bacteria colonies.

Four test tubes were placed in the incubator with extracted tooth samples and artificially prepared cariogenic medium was added. (Fig. 25) The teeth were retrieved once every 24 hours and washed with the help of syringe with 5 mL of the anticariogenic study solutions for 21 days. After 21 days, the teeth were retrieved from the anticariogenic solution and prepared for demineralization depth studies. (Fig. 26)

Demineralising Teeth
For demineralising the tooth were immersed in 15percent of Nitric acid. The nitric acid was prepared by mixing 82ml of distilled water in 18ml of nitric acid. The concentration of nitric acid is 60-70.

Preparation of Ground section
The ground section is prepared by cutting the teeth using the diamond disk burr. After thinning the teeth, the teeth are shredded over the arkinsa stone. When the teeth become paper thin, the teeth were fixed with glycerine and to lab for polarised light microscopy.

Evaluation of Ground Section
All the samples were taken and analysed under the stereo type microscope. All the depth of the caries was evaluated using the morphometry lens and the value measured were measured in the micrometer.

STATISTICAL ANALYSIS
The data for the present study was entered in the Microsoft Excel 2007 to prepare masterchart and analyzed using the SPSS statistical software 23.0 Version. The descriptive statistics included mean, standard deviation. The intragroup comparison for the different time intervals was done using Repeated Measures ANOVA to find the difference between the individual time intervals The level of the significance for the present study was fixed at 5%.

The intergroup comparison for the difference of mean scores between two independent groups was done using the One Way ANOVA followed by Post Hoc Analysis

The Shapiro–Wilk test was used to investigate the distribution of the data and Levene's test to explore the homogeneity of the variables. The data were found to be homogeneous and normally distributed. Mean and standard deviation (SD) were computed for each variable

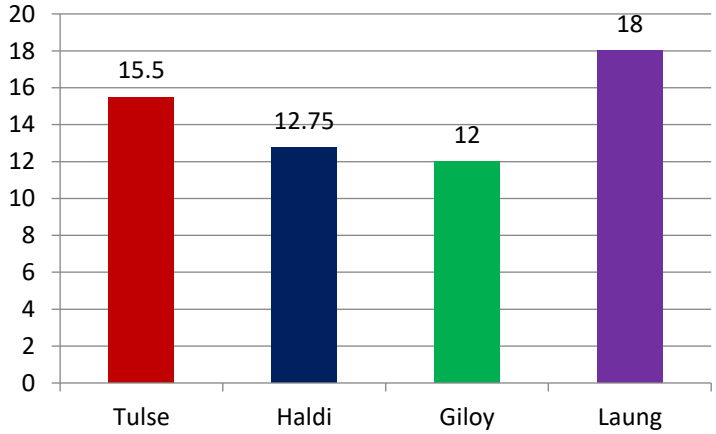
Observation & Result
The zone of inhibition in the culture medium, with respect to specific herbal extracts, with the measure of extent, happened to be the key feature in evaluating the effectiveness of the extracts in terms of bacteria which was done in phase I of the current study.

FOR LACTOBACILLUS ACIDOPHILUS:
At 24 hours, the mean zone of inhibition for the Lactobacillus in terms of the Tulsi extracts was 15.50 mm, in terms of the Haldi extracts it was 12.75 mm, for the Giloy extracts it was 12.00 mm which happened to score the least amount of inhibition zone and largest zone of inhibition was for the Laung extracts (18.00mm)

The difference between the groups was statistically significant when analyzed using One Way ANOVA at p value of less than 0.039.

Post Hoc Analysis

	Mean Diff	Std Error	P value
Tulsi vs Haldi	2.75000	1.95523	0.185 (Non-Significant)
Tulsi vs Giloy	3.50000	1.95523	0.099 (Non-Significant)
Tulsi vs Laung	-2.50000	1.95523	0.225 (Non-Significant)
Haldi vs Giloy	.75000	1.95523	0.708 (Non-Significant)
Haldi vs Laung	-5.25000*	1.95523	0.020 (Significant)
Giloy vs Laung	-6.00000*	1.95523	0.010 (Significant)

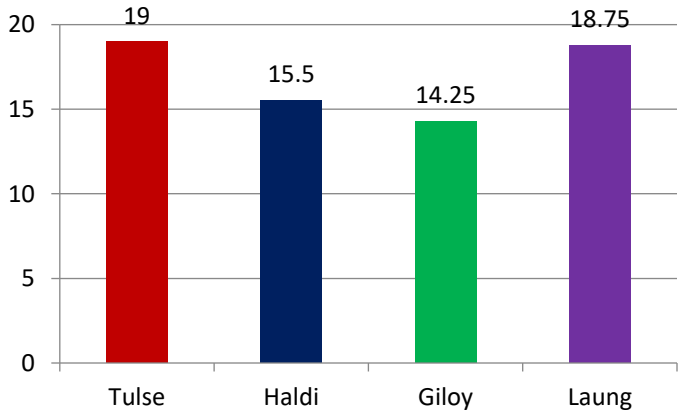


Graph 1: showing inhibition zone-at 24 hrs

At 48 hours interval, the mean zone of inhibition for the Lactobacillus in terms of the Tulsi extracts was 19.00 mm that was happened to be largest, for the Haldi extracts it was 15.50mm, for the Giloy extracts it was 14.25mm which was minimum and second largest zone of inhibition was for the Laung extracts (18.75mm).

Post Hoc Analysis

	Mean Diff	Std Error	P value
Tulsi vs Haldi	3.50000	2.64969	0.211 (Non-Significant)
Tulsi vs Giloy	4.75000	2.64969	0.098 (Non-Significant)
Tulsi vs Loung	.25000	2.64969	0.926 (Non-Significant)
Haldi vs Giloy	1.25000	2.64969	0.646 (Non-Significant)
Haldi vs Loung	-3.25000	2.64969	0.244 (Non-Significant)
Giloy vs Loung	-4.50000	2.64969	0.115 (Non-Significant)

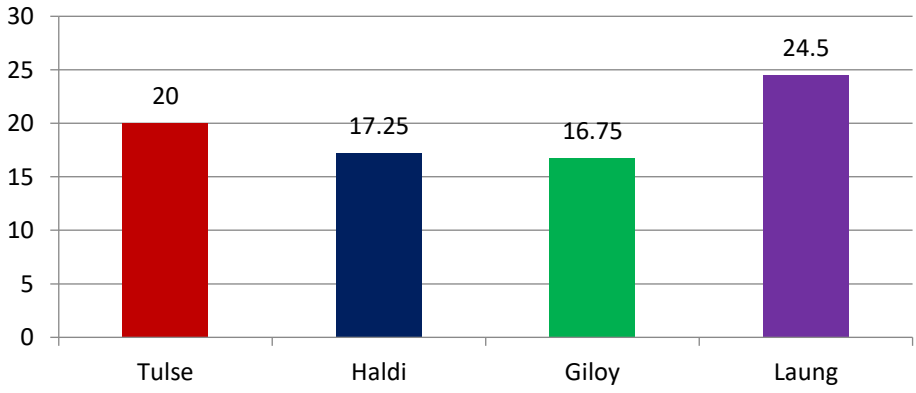


Graph 2: Zone of inhibition in lactobacillus is 48 hours

After ,72 hours the mean zone of inhibition of the Tulsi extracts for the Lactobacillus was 20.00mm, for the Haldi extracts it was 17.25mm, for the Giloy extracts it was 16.75mm that was minimum of the zones and largest zone of inhibition was for the Laung extracts (24.50mm). The difference between the groups was statistically significant when analyzed using One Way ANOVA at p value of less than 0.049.

Post Hoc Analysis

	Mean Diff	Std Error	P value
Tulsi vs Haldi	2.75000	3.03109	0.382 (Non-Significant)
Tulsi vs Giloy	3.25000	3.03109	0.305 (Non-Significant)
Tulsi vs Loung	-4.50000	3.03109	0.163 (Non-Significant)
Haldi vs Giloy	.50000	3.03109	0.872 (Non-Significant)
Haldi vs Loung	-7.25000*	3.03109	0.034 (Significant)
Giloy vs Loung	-7.75000*	3.03109	0.025 (Significant)



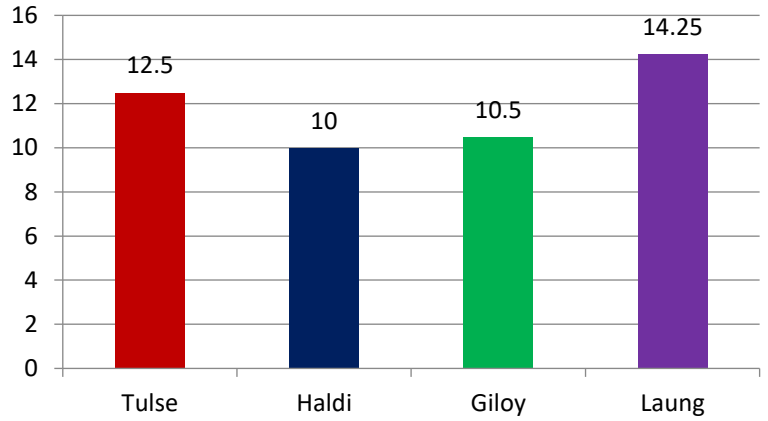
Graph 3: Maximum inhibition zone shown by the laung at 72 hours

FOR STREPTOCOCCUS MUTANS:

At 24 hours for S. Mutans of inhibition for the zone of inhibition of the Tulsi extracts was 12.50 mm, for the Haldi it was 10.00 mm, for the Giloy it was 10.50 mm which was having minimum score and largest zone of inhibition was for the Loung (14.25mm). The difference between the groups was statistically significant when analyzed using One Way ANOVA at p value of less than 0.001

Post Hoc Analysis

	Mean Diff	Std Error	P value
Tulsi vs Haldi	2.50000*	.88388	0.015 (Significant)
Tulsi vs Giloy	2.00000*	.88388	0.040 (Significant)
Tulsi vs Loung	-1.75000	.88388	0.049 (Significant)
Haldi vs Giloy	-.50000	.88388	0.582 (Non-Significant)
Haldi vs Loung	-4.25000*	.88388	0.001 (Significant)
Giloy vs Loung	-3.75000*	.88388	0.001 (Significant)

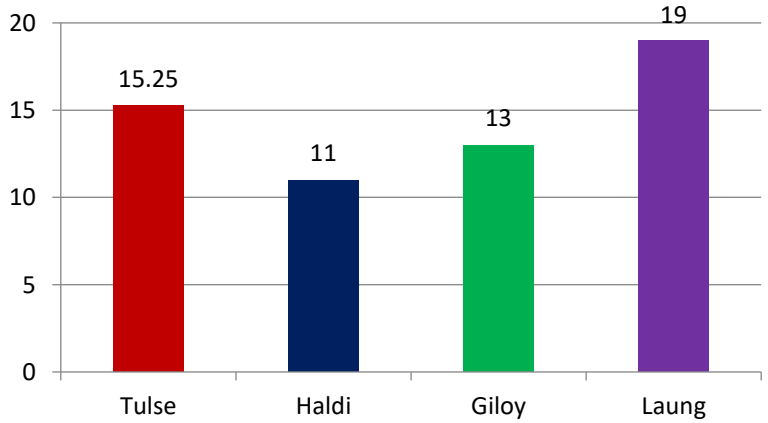


Graph 4: Zone of inhibition in streptococcus is 24 hours

At 48 hours interval for S.Mutans the mean zone of inhibition in terms of the Tulsi extracts was 15.25mm, for the Haldi extracts it was 11.00mm, for the Giloy extracts it was 13.00 mm and largest zone of inhibition was for the Laung extracts (19.00mm).

Post Hoc Analysis

	Mean Diff	Std Error	P value
Tulsi vs Haldi	4.25000*	.78395	0.001 (Significant)
Tulsi vs Giloy	2.25000*	.78395	0.014 (Significant)
Tulsi vs Laung	-3.75000*	.78395	0.001 (Significant)
Haldi vs Giloy	-2.00000*	.78395	0.055 (Non-Significant)
Haldi vs Laung	-8.00000*	.78395	0.001 (Significant)
Giloy vs Laung	-6.00000*	.78395	0.001 (Significant)

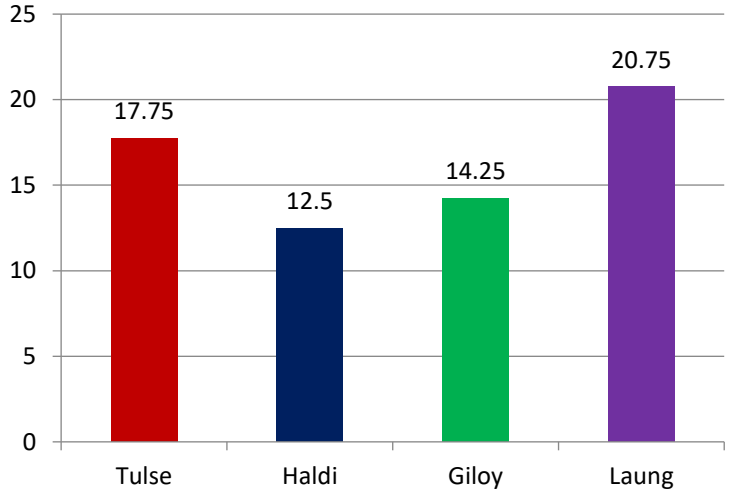


Graph 5: Zone of inhibition streptococcus is 48 hours

At 72 hours the mean zone of inhibition for S.Mutans in terms of the Tulsi extracts was 17.75, for the Haldi extracts it was 12.50 that was minimum, for the Giloy extracts it was 14.25 and largest zone of inhibition was for the Laung (20.75). The difference between the groups was statistically significant when analysed using One Way ANOVA at p value of less than 0.001.

Post Hoc Analysis

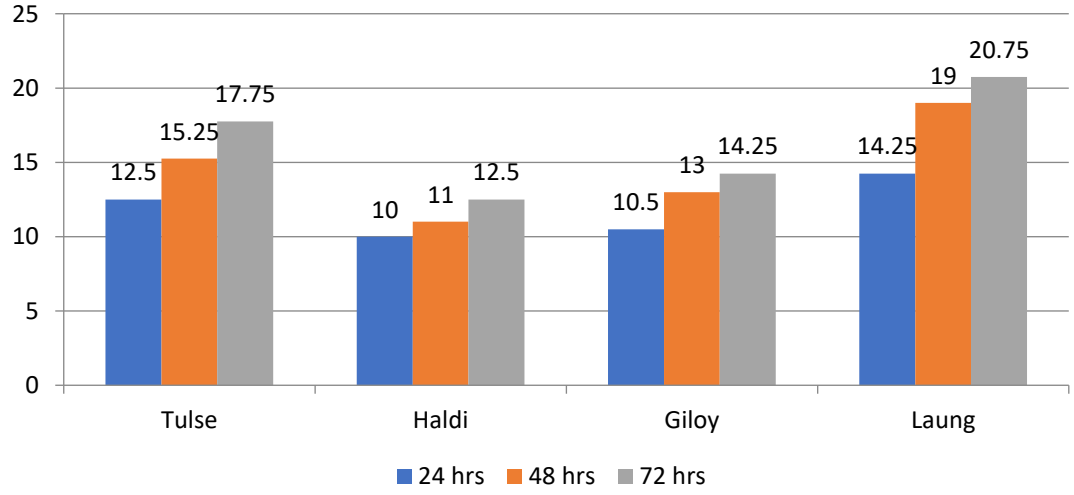
	Mean Diff	Std Error	P value
Tulse vs Haldi	5.25000*	1.02571	0.001 (Significant)
Tulsi vs Giloy	3.50000*	1.02571	0.005 (Significant)
Tulsi vs Loung	-3.00000*	1.02571	0.013 (Significant)
Haldi vs Giloy	-1.75000	1.02571	0.114(Non-Significant)
Haldi vs Loung	-8.25000*	1.02571	0.001 (Significant)
Giloy vs Loung	-6.50000*	1.02571	0.001(Significant)



Graph 6: Zone of inhibition in streptococcus in 72 hours

Table 7: INTRAGROUP COMPARISON OF THE S.MUTANS BETWEEN 24HRS-48HRS AND 72 HRS IN FOUR GROUPS

	24 hrs	48 hrs	72 hrs	P value
Tulsi	12.5000	15.2500	17.7500	0.001 (Sig)
Haldi	10.0000	11.0000	12.5000	0.001 (Sig)
Giloy	10.5000	13.0000	14.2500	0.001 (Sig)
Laung	14.2500	19.0000	20.7500	0.001 (Sig)



Graph 7: Comparative analysis of all the extracts

In phase II of the study, ground section samples were evaluated by the depth of carious lesion with respect to herbal extracts which were demarcated by different colours for the sake of easement , as for the tulsi extracts, the samples were colour coded in red , the haldi was coded with green, laung was marked as blue and Giloy with yellow. Then the samples are evaluated by using the stereo microscope. The mean depth of the caries for the Tulsi extracts was 870.00 micrometer, for the Haldi extracts it was 1225.03 micrometer, for the Giloy extracts it was highest 1430.03 micrometer and smallest caries depth was for the Loung extracts as 440.00 micrometer.

Table 8: INTERGROUP COMPARISON BETWEEN FOUR GROUPS FOR THE DEPTH OF CARIOUS LESION

	Mean	Std. Deviation	Std. Error	Minimum	Maximum	P value
Tulsi	870.00	122.927	38.873	700.00	1100.00	0.001(Significant)
Haldi	1225.03	145.773	46.097	1000.00	1450.00	
Giloy	1430.03	204.396	64.635	1100.00	1800.00	
Laung	440.00	61.463	19.436	350.00	550.00	

Post Hoc Analysis

	Mean Diff	Std Error	P value
Tulsi vs Haldi	-355.00000*	63.99870	0.001 (Significant)
Tulsi vs Giloy	-560.00000*	63.99870	0.001 (Significant)
Tulsi vs Loung	430.00000*	63.99870	0.001 (Significant)
Haldi vs Giloy	-205.00000*	63.99870	0.001 (Significant)
Haldi vs Laung	785.00000*	63.99870	0.001 (Significant)
Giloy vs Laung	990.00000*	63.99870	0.001 (Significant)

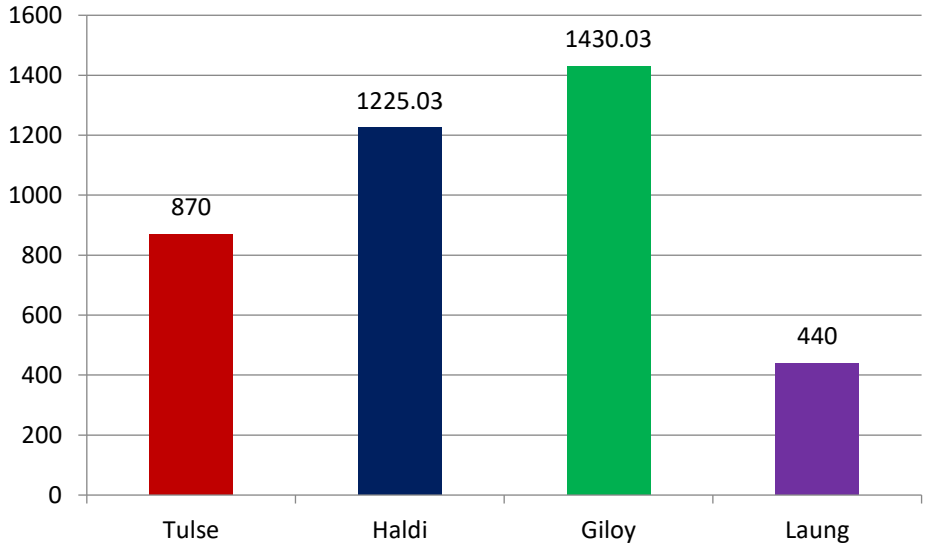


Table 8: Comparison of Depth in caries

DISCUSSION

For the prevention of these diseases, we can focus on increasing the ability of the host, decreasing the cariogenicity of the bacteria, and promoting less cariogenic diet. Therefore, for proper caries control, various methods in combination should be followed with chemoprophylactic agents. Thus, what we require is a safe, effective, and economical treatment plan for the prevention of dental caries. Synthetic drugs have many adverse effects, so main focus should be toward natural remedies which are safe as well as effective.[4]

For this study, we evaluated the four herbal extracts (*Ocimum Sanctum* (Tulsi), *Curcuma longa* (Turmeric), *Syzygium aromaticum*(Laung), and *Tinospora cordifolia* (Giloy)). The extracts that were taken for the study were made water soluble and propylene glycol soluble with Ph between 4-5. According to Padmawar AR et al in 2018, propylene glycol is widely used in mouthwash, toothpaste, industrial cleaners and as antifreeze liquid. To give hydrophilic nature to plant extracts (hydroglycolic extracts) we require utilization of propylene glycol. The advantages offered by the

glycol is that Glycols offer slightly sweet scent and neutral colour. It possess chemical stability, long shelf-life and act as good preservative as well.[47]

The results of our phase I study shows that the Laung is the most potent liquid extracts against both the bacterias i.e. *Streptococcus mutans* and *Lactobacillus acidophilus*. The zone of inhibition was larger in L.acidophilus as compared to the S.mutans. Similar study was conducted by Aneja et al. in 2010 which showed that the clove and clove oil have potent antimicrobial activity against the cariogenic microorganisms. Clove oil showed the highest zone of inhibition of 34.32 mm against S.mutans, which was even much higher than the positive control i.e. ciprofloxacin (27.32mm),S.mutans was found to be most sensitive pathogen which survived upto 1.56 mg/ml in clove oil which is in accordance to our study.[48] Similar results are also produced by the study done by Elgamily H et al. in 2019 in which clove produced inhibition zones against Streptococcus mutans and Lactobacillus acidophilus growth. MIC for the plant showed equal antimicrobial activity against Streptococcus mutans, while Clove had a higher sensitivity to Lactobacillus acidophilus.[49]

Syzygium aromaticum(Laung) as compared with other herbal liquid extracts, i.e. *Ocimum Sanctum* (Tulsi), *Curcuma longa* (Turmeric) and *Tinospora cordifolia* (Giloy), in study done by Shrivastava A et al in 2019, when compared inhibitory properties against the E.coli, *S. aromaticum* oil(Laung) showed maximum and *O. sanctum* (Tulsi) fresh leaf juice showed minimum inhibitory potential and percent inhibition against ESBL enzymes. The combination of "Laung" and "Tulsi" showed better activity than that of individual herbs. The difference in the observations could be due to the color of the herbs, which may have caused the hindrance in the absorbance value, giving a low percentage of inhibition rate. A crucial advantage of a colorimetric method is that change in color can be directly detected. Herbs alone or in combination can be an improved substitute to the antibiotics for the poultry and other livestock sectors which is also in favour of our study.[50]

For the Phase II of the study, the lowest depth of the caries is seen in case of "Laung". The laung prevent caries and resist tooth from decay. Our result is been similar to the study conducted by Verma SK et al in 2017 stated that chewing of cloves diminishes bad breadth. The microorganisms which cause bad breath are chosen from the group consisting of: Eubacterium, Fusobacterium, Haemophilus, Neisseria, Porphyromonas, Prevotella, Treponema and Veillonella species. Eugenol acetate present in clove oil is used for inhibiting the growth of microorganisms which cause and combat bad breath. Clove helps to decrease infection due to their antiseptic properties. Eugenol is the most important compound of dianthus essential oil with strong antibacterial and anaesthetic properties. Eugenol shows strong destructive effect on viruses, bacteria, saccharomycetes, moulds and protozoans. A very important characteristic of Eugenol is presence of essential oil in it is its activity against microorganisms resistant to synthetic antibiotics.[51]

In Phase I of the study the "Tulsi" emerges to be one of the second best anti-microbial extract after the "Laung". Similar study conducted by Gadiyar A et al. in 2017, where they stated that all the parts of *Ocimum sanctum* L that is leaves, stem, flower, root, seeds are known to have several therapeutic potentials and have been used as analgesic, antimicrobial, anticancer, anti-asthmatic, anti-diabetic, anti-fertility, anti-spasmodic, hepatoprotective, antiemetic, cardioprotective and antistress agents. Ocimum sanctum L. fixed oil has shown good antibacterial activity against Staphylococcus aureus, Bacillus pumius and Pseudomonas aeruginosa. Higher content of linoleic acid in Ocimum sanctum L. fixed oil could also contribute towards its antibacterial activity.[2] Geeta et al. in 2001 studied that the aqueous extract of O. sanctum L. (60 mg/kg) show wide zones of inhibition compared to alcoholic extract against Klebsiella, Escherichia coli, Proteus, Staphylococcus aureus and Candida albicans when studied by agar diffusion method. Alcoholic extract showed wider zone for Vibrio cholera. Extract of Ocimum sanctum caused inhibition of both, Neisseria gonorrhoeae clinical isolates and WHO organization strains.[52]

For the phase II of the study our results were similar to Gupta B et al. in 2013, the MIC against Streptococcus mutans was found to be 4% and 6% respectively by cup and plate method. The variation may be due to the fact that the chemical constituents may vary due to edaphic and geographic factors, difference in the microbiological method used and variation in the solvent used to prepare the extract. The minimum inhibitory concentration of Ocimum sanctum against Lactobacillus acidophilus was determined to be 10% (100mg/ml). [53]

As some previous studies has also stated that *Ocimum sanctum* is not always affective against all the oral microorganism. According to Yamani et al.

in 2016 stated that the antibacterial efficacy of "Tulsi" i.e. *Ocimum sanctum* oil has lowered the activity of *S. aureus*, MRSA and *E. coli* but *P. aeruginosa* showed higher resistance to the antibacterial treatment with Tulsi oil.[40]

As compared with "Giloy" and "Haldi" the *Ocimum sanctum* (Tulsi) shows better zone of inhibition at lower concentration. Similar results were shown by Mistry KS et al. in 2014, which confirmed significant antimicrobial activity in which *O. sanctum* (Tulsi) and showed highest zone of inhibition against *S. mutans* at 3 mg concentration whereas *T. cardifolia* (Giloy) showed no inhibitory effect at lower concentration.[54]

As the phase I of the study shows that "Haldi" *Curcuma longa* shown the little level of antibacterial activity as compared to "Laung" and "Tulsi". Our results been compared with Islam TH et al. in 2012 showed turmeric to be effective in inhibiting growth of Streptococcus mutans to an appreciative level. The crude extract of turmeric, 100 mg/ml of pH 4.5 showed a zone of inhibition of 18 mm. While at pH 7.0, the zone was 17 mm indicating that the ethanolic content of the extract had no impact on the pH. No MIC or MBC was done for the combination of the herbal agents. The effectiveness of turmeric is found to be different at different concentration and solvent used with it.[55] According to Lee KH et al. in 2011, showed the ability of C. longa essential oil to inhibit the bacterial growth, acid production, adherence to HAs, and biofilm formation of S. mutans.[56]

The higher concentration of "Haldi" (Curcuma longa) plays an important role in caries reduction activity. The phase II of the study, shows the same activity as phase I. Our study somewhat resembles the result of the Tyagi P et al in 2015, they observed a 100% killing at a dosage of 100 µM curcumin even when higher bacterial density (10^6 CFU/ml) was used. There observations were in agreement with previous studies, which showed an 80% decrease in *E. coli* cell growth on exposure of 100 µM curcumin, 100% inhibition of *S. aureus* and *P. aeruginosa*, and 80% inhibition in case of *E. faecalis* due to the exposure of 200 µM of curcumin within four hours. The high concentrations required to kill the bacteria in this and other studies may be due to poor solubility of curcumin in aqueous media and the low bioavailability of curcumin.[57]

The herbal extract in our study with least antibacterial activity is "Giloy" i.e *Tinospora cordifolia*. The inhibition activity of Giloy against *Streptococcus mutans* and *Lactobacillus acidophilus* is highly questionable. Similar results are observed by the Mistry KS in 2014, Giloy also exerted considerable antimicrobial effectiveness against tested pathogens. However, it is ineffective against E. faecalis and S. aureus at lower concentrations with MIC value of 500 µg. This plant has been subjected to chemical investigations extensively and a number of chemical constituents belonging to different groups such as terpenoids, alkaloids, lignans and flavonoids, tannins, cardiac glycosides and steroids have been reported. which may account for the antimicrobial property of this agent.[55] According to Vermani A et al. in 2013, concluded that T. cordifolia produced the least inhibitory effect and maximum decay depth when compared to G. glabra (Licorice), O. sanctum (Tulsi), and T. chebula (Harad) against S. mutans and L. acidophilus studied at all-time intervals. Tinospora cordifolia is a potent antimicrobial agent. This may be attributed to the fact that T. cordifolia only contains alkaloids, flavonoids, steroids, tannins, phenols, and saponins.[58]

Our study is highly contradicted by the study conducted by Prasad B et al. in 2019. In his study, they showed that the antimicrobial activity of ethanolic and methanolic extract of root and stem of T. cordifolia were also evaluated against some pathogenic microorganisms viz. E. coli, B. subtilis, A. niger and Candida sp in which various concentration of extract viz. 50, 100, 150 and 200 mg ml-1 were tested.

It was observed that the increase in concentration increases the antimicrobial activity revealed by increase in size of zone of inhibition. The methanolic stem extract exhibits highest antimicrobial activity against all four pathogens.[59] This study shown that the extract of T. cordifolia has a wide range of anti-oxidant as well as antimicrobial activity against bacterial as well as fungal pathogens.

These plants are well documented in ancient literature about their effectiveness. No doubt this could be the reason that they are a important part in most of the kitchens as our ancestors were having farsighted and they insist on incorporating these ayurvedic medicines directly to the kitchen.

With introduction of scientific procedures, the researchers have concluded that the plants contain active principles responsible for curative action of the herbs. After reviewing, there is considerable evidence that plants extracts, and purified

phytochemicals have potential to be developed into agents which can be used as a preventive or treatment therapies for oral diseases such as dental caries and periodontal problems. These medicines will be cost effective and range in many common people. Therefore, more in-vivo studies need to be done over this subject to have the better knowledge and applicability of these gift of nature as there are numerous extracts which yet to be studied.

CONCLUSION

In the first phase of the study,
Laung (*Syzygium aromaticum*) showed the largest inhibitory zones on microbial growth against S. mutans and L. acidophilus studied at all-time intervals as compared to any other herbal extracts.

Followed by the Laung, *Ocimum sanctum* (Tulsi) showed the second largest inhibitory zone. Ocimum sanctum is used since ages for the antibacterial and antioxidant activity. Since, the antibacterial activity of the Tulsi is still appreciable.

After Tulsi, the inhibitory zone showed by Curcuma longa (Haldi) is more than that of Giloy, but it would be too soon to call Haldi as less potent anticariogenic as many previous studies had revealed the antimicrobial activity of "haldi" at different concentrations.

Lastly, T. cordifolia (Giloy)showed the least inhibitory zone in the study with hardly any antimicrobial activity.
In the second phase of the study,

The results after measuring the overall decay depth of *Tinospora cordifolia* (Giloy) reveals that the Giloy was hardly able to prevent caries as compared to other herbal extracts. Giloy showed quite high decay depth.

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