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Evaluation of the anti-microbial properties of prepared herbal solution on dental impressions with irreversible hydrocolloid- an ex vivo study

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Abstract
Aim: of this study is evaluation of the anti-microbial properties of prepared herbal solution on dental impressions with irreversible hydrocolloid. The study is an ex-vivo comparative study.
Material and methods: includes Herbal ingredients: Neem, Tulsi, Reetha, Aloe vera, Neem seed oil, Ginger oil, Peppermint oil, Gum acacia, and Rock salt were used to prepare herbal disinfectant solution. Preparation of this disinfectant solution includes extract preparation by reflux method, filtration, evaporation and homogenization. Minimum inhibitory concentration of this solution was determined and in-vitro antimicrobial properties were checked with disc diffusion method. To check whether the anti-microbial property of herbal solution was useful in dentistry, 10 sectional maxillary impressions of left side were made with irreversible hydrocolloid. In the molar area each impression was cut in two halves. One half was washed with water and other half was sprayed with herbal solution. Impressions were kept for 2 to 3 minutes and then placed in inoculated agar plates. Incubation of agar plates was done for 24 hours and checked for bacterial growth. Mann Whitney 'U' test and Chi Square test were used to evaluate the data.
Results: found that Agar plates having sectioned alginate impression sprayed with disinfectant herbal solution showed less bacterial growth compared with the agar plates having alginate washed with water. To conclude Herbal disinfectant solution is effective in controlling the bacterial growth on impressions with irreversible hydrocolloid.

Keywords: Impressions, herbal disinfectant, agar plates, aloe vera, neem, tulsi

1. Introduction
Microbes appear in every corner of human life and affect every aspect of human life. The human oral cavity contains a number of different habitats. Synergy and interaction of variable microorganisms help human body against invasion of undesirable stimulation outside. However, imbalance of microbial flora contributes to oral diseases and systemic diseases [1]. Approximately 280 bacterial species from the oral cavity have been isolated [2]. The importance of cross-infection control cannot be overemphasized. Disinfection and sterilization methods are used to achieve disinfection and sterility of the medical and surgical instruments. In order to avoid the spread of pathogens from patients to patient, patient to health care personnel and health care personnel to patient, it is the duty of the health care policy makers to allocate the appropriate methods of cleaning, disinfection and sterilization for various surfaces and instruments [3].
Similarly, when oral impression is made there is a high risk of transferring these bacteria from the patient's oral cavity to the dentist, dental assistant and laboratory personal. It is not sufficient to wash it with water alone. Thorough cleaning with water alone is inadequate in eliminating the oral pathogens. The impression can be disinfected by two methods
1. Immersion in disinfectant and
2. Spraying with a disinfectant agent.

Immersion technique is more efficient in eliminating the microorganisms from the surface of the impression. But the disadvantage of immersion technique is that it leads to alteration in the surface of the impression which can lead to loss of detail reproduction. Spraying method doesn't alter the surface characteristics of the impression [4].

There are many disinfectant chemicals available as solution or spray for dental impressions in the market. These chemicals contain aldehydes, alcohols, phenols, chloride, iodine combinations, ammonium compounds and biguanides. These are synthetic chemicals which could cause deleterious effect on human health [5].

Organic and natural products with efficient disinfectant property would be a non-toxic appro-ach to prevent cross infection. In this study we have prepared an herbal solution with different natural herbs and checked its antimicrobial property.

2. Materials and Methods

The study was approved by institutional ethical committee and the study was conducted abiding by all human ethical principles as per the WMA- Declaration of Helsinki and the Guidelines of Good Clinical Practice (ICMR) were followed.

This study was done in two parts -

I) *In vitro* study which included 1) preparation of herbal extract 2) finding minimum inhibitory concentration and zone of inhibition by disc diffusion method.

II) *In vivo* study which included 1) culturing bacteria and then finding effect of herbal spray on the bacteria under microscope.

Objectives of the study

1) Antimicrobial property – study of antimicrobial property was done in terms of microbial count.
2) Smear layer assessment

Materials used for the study

Herbal ingredients - Neem, Aloe Vera, Tulsi, Reetha, Rock salt, Peppermint oil, Ginger, Neem seed oil and Gum acacia.

Instruments for herbal solution preparation – All the apparatus required for reflux method, mortar, pestle, filter funnel, filter paper, muslin cloth, porcelain dish, homogenizer and amber color reagent bottle.

Materials and instruments for *in vivo* study – Alginate, impression trays, cutter, herbal solution, agar plates, smear plates and microscope.

I) *In vitro* study which includes

Before starting the preparation, authentication of all the ingredients used for herbal preparation was done. [Fig.9]

1) Preparation of herbal extract by reflux method [Fig.1]

Neem, Tulsi and Reetha were taken in powder form along with their solvents and placed in a round bottom flask for condensation process for one and half hours. After which solution was cooled for one hour, then this solution was filtered with muslin cloth. [Fig.2, Fig.3] The filtrate obtained was evaporated to get the extract. [Fig.4]

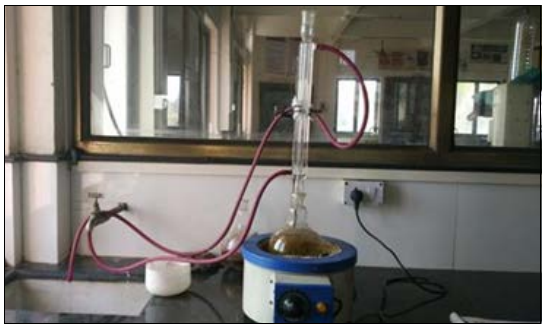

Fig 1: Reflux method

Fig 2: Formed Solution

Fig 3: Filtration

The remaining ingredients were used in their raw form. The extracts obtained from the above method were measured with other ingredients and triturated one by one. [Fig.5, Fig.6] Prepared mixture was homogenized with homogenization machine. [Fig.7] The solution obtained after homogenization was poured in amber color reagent bottle and checked for easy spraying [6]. [Fig.8]

Fig 5: Ingredients calculated

Fig 6: Trituration

Fig 7: Homogenization Machine.

Fig 8: Herbal spray

Fig 9: Authentication of ingredients

Fig 10: Alginate impression

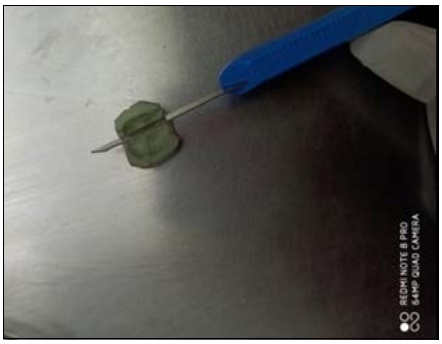

Fig 11: Sectioned in two parts

Fig 12: one part washed with water and other part sprayed with herbal solution

2) Finding antimicrobial activity by – Minimum inhibitory concentration and Disc diffusion method.

At what concentration prepared herbal solution was bactericidal and fungicidal against S. mutans and Candida albicans was found out in lab by minimum inhibitory concentration with nine serial dilutions.

The concentrations that were found out to be bactericidal were used for disc diffusion method. In disc diffusion method on inoculated culture media of S. mutans and Candida albicans five equal wells of five different concentration of herbal solution were poured and incubated. After 24 hours zone of inhibition was seen [7].

II) *In vivo* study was carried out in the following steps

10 patients in the age group of 20-40 years visiting the outpatient department of Prosthodontics, Crown and Bridge were included in the study.

Patients with any systemic disease or salivary gland pathology were excluded from the study.

Herbal solution was tested on irreversible hydrocolloid impression of the patients. Sectional impressions on the left maxillary arch were made in irreversible hydrocolloid. [Fig.10] Each impression was sectioned into two parts at molar area. [Fig.11] One part of the impression at the molar area was cleaned with tap water and other part was sprayed with antimicrobial herbal solution. [Fig. 12] For culturing these sectional impressions four agar culture media were used. Two agar plates used for checking growth of bacteria after spraying with herbal solution and two agar plates used for checking growth of bacteria after washing with water. As 10 samples were used agar plates for herbal and water were divided into 10 sections and marked from X_1 to X_{10}. 10 sectioned samples of each group were placed into their respective area on agar plates after spraying and washing with herbal solution [Fig.13] and water [Fig.14] respectively. This agar plates were kept for incubation for 24 hours. After 24 hours samples were taken out carefully from incubator. 20 glass smear plates were taken and marked X_1 to X_{10} for herbal samples and X_1 to X_{10} for water samples. With the help of a sterilized ear bud the swabs of the 2mm area around each impression on agar plate were taken and sprayed on a respective glass smear. Glass smear were stained and kept for 30 to 60 seconds. Each glass smear was checked under microscope visually. [Fig.15]

Two main parameters 1) Antimicrobial property was measured in terms of microbial count. It was a quantitative continuous variable so expressed as mean and standard deviation. As the data obtained did not have normal distribution, non-parametric Mann Whitney test was used to assess the data. 2) Smear layer was a categorical binary variable and measured in terms of present or absent. The data obtained was in percentage so chi square test was used to analyze the data.

Fig 13: Without spray

Fig 14: with spray

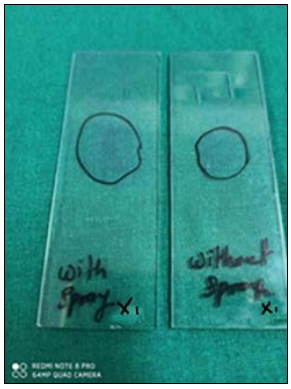

Fig 15: Growth of the bacteria on smears

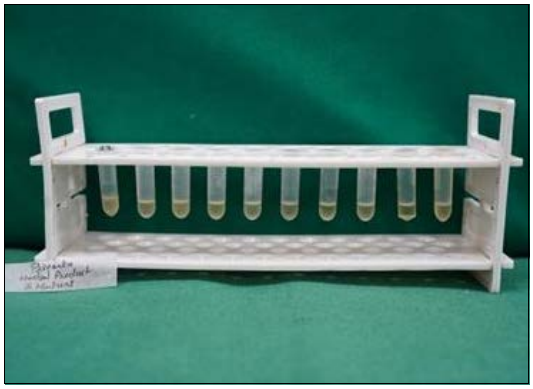

Fig 16: MIC of S. Mutans

Fig 17: MIC of C. Albicans

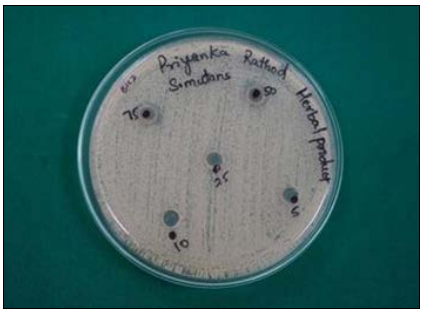

Fig 18: Zone of inhibition of S. Mutans

Fig 19: Zone of inhibition of C. Albicans

Fig 20: Smear plate under microscope

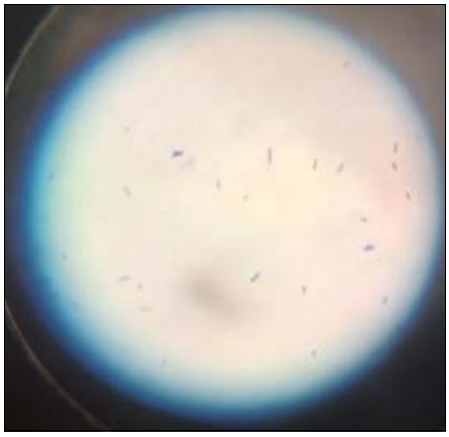

Fig 21: Bacteria growth without spraying

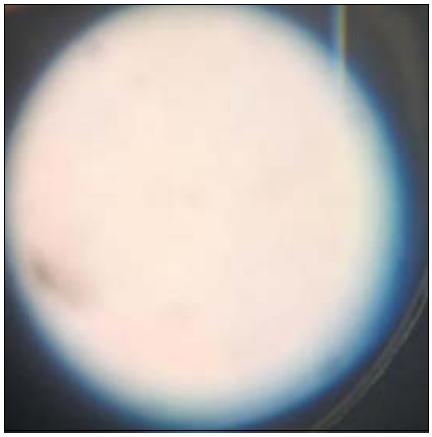

Fig 22: Bacteria growth with spraying

3. Result

The antimicrobial concentration of herbal solution was found out with minimum inhibitory concentration method (MIC)[Fig 16 & 17] The disc diffusion method was used to find out the zone of inhibition of streptococcus mutans and candida albicans.[Fig 18 &19]

Table 1: MIC

Sl. No.	Samples	100 µl/ml	50 µl/ml	25 µl/ml	12.5 µl/ml	6.25 µl/ml	3.12 µl/ml	1.6 µl/ml	0.8 µl/ml	0.4 µl/ml	0.2 µl/ml
Herbal Product											
1	S.mutans	S	S	R	R	R	R	R	R	R	R
2	Candida albicans	S	S	S	S	S	S	R	R	R	R

Table 2: Disc Diffusion Results

Sl. No.	Samples	75µl/ml	50µl/ml	25µl/ml	10µl/ml	5µl/ml
Herbal Product						
1	S.mutans	13mm	12mm	R	R	R
2	Candida albicans	12mm	10mm	R	R	R

S – Sensitive, R – Resistant

Standard values for MIC Fluconazole: C.albicans- 16µg/ml, Ciprofloxacin: S.mutans- 2ug/ml

Standard values for disc diffusion Fluconazole: C.albicans - 24mm, Ciprofloxacin: S.mutans-26mm

Further we studied the anti-microbial effect of this solution in the oral cavity. For that irreversible hydrocolloid impression were made and cut into two halves. One half was washed with water and other half was sprayed with herbal solution and incubated on agar plates.

Smear obtained from this sample was checked under microscope. [Fig. 20] Result showed that bacterial growth was seen in sample without spray [Fig. 21] and less growth was seen with sample sprayed with herbal solution. [Fig. 22]

Table 3: Quantification of microorganisms showing mean bacterial count with presence and absence of herbal disinfectant solution.

	Mean	S.D	Mann Whitney 'U' TEST	p value, Significance
With herbal disinfectant solution (n=12)	0.3 x 10^6	0.08 x 10^6	U =6.912	*p* <0.001**
Without herbal disinfectant solution (n=12)	458 x 10^6	139 x 10^6		

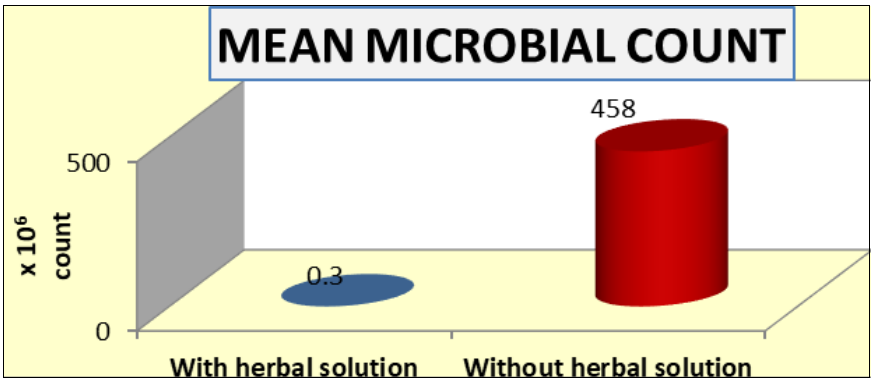

Fig 23: Mean microbial count

From the above table and graph it is clear that herbal disinfectant solution significantly reduces bacteria compared to water.

Table 4: Comparison of presence of bacterial smear layer with & without disinfecting with herbal disinfect

	Presence of bacterial smear	Chi square test	p value, Significance
With herbal disinfect solution (n=12)	3/12 (25%)	Chi = 24.61	p = 0.002*
Without herbal disinfect solution (n=12)	8 /12 (66.6%)		

p <0.05 – significant difference ** *p* <0.001 – highly significant difference.

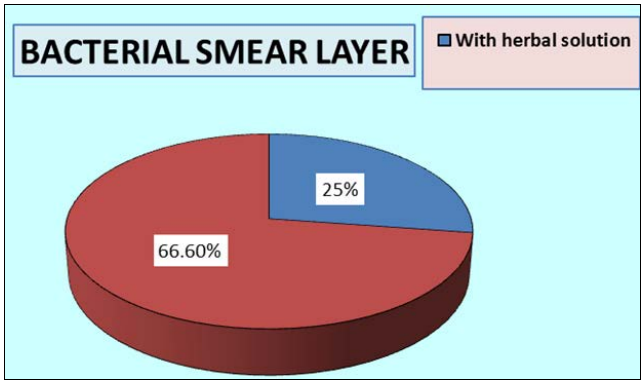

Fig 24: Bacterial smear layer

From above table and graph we can appreciate that the smear layer of colonies formed around irreversible hydrocolloid impression on agar plate sprayed with herbal disinfectant solution were significantly reduced, whereas the smear layer of colonies formed around irreversible hydrocolloid impressions on agar plate washed with water had more bacterial count.

4. Discussion

Plaque contains hundreds of different species of microorganisms in a matrix of host salivary molecules and bacterial extracellular products. If the plaque is successfully cleaned away then the cycle begins again. However, plaque that is inaccessible to removal for various reasons forms the basis for polymicrobial diseases of the oral tissues and, if systemically disseminated, form diseases of the cardiovascular, musculo-skeletal and nervous systems [2].

Alginate is an elastic, irreversible hydrocolloid impression material. Irreversible hydrocolloid impressions form an inseparable part of indirect restorations. They form a major bulk of our clinical practice even today [8]. Therefore Irreversible hydrocolloid (Alginate impressions) act as a vehicle for transmission of microorganisms.

In the study done by Hamid Badrian and colleagues in the year 2012, stated that simple rinsing with water would apparently not remove all blood and saliva due to the presence of salivary adhesive proteins and salivary mucins on the impression surface. Hazardous microorganisms like Staphylococcus aureus, Methicillin resistant Staphylococcus, Candida albicans and Pseudomonas aeruginosa are present with rate of 55.6%, 25.9%, 25.9%, and 5.6%, respectively. There is always a high risk for transfer of opportunistic pathogens from oral cavity to alginate impression. Hence when we handle or transfer these impressions there is a higher chance of transferring all these bacteria to doctors as well to the technicians [9].

They mentioned that use of commonly available chemical disinfectants like sodium hypochlorite reduced the microbial count by 99.9% when compared to rinsing with water which is 48.5% [9]. In our study the bacterial count present when sprayed with herbal disinfectant solution was 25% whereas bacterial count present when washed with water was 66.60% [9].

Different disinfection procedures and immersion times affect the dimensional accuracy or surface detail of an irreversible hydrocolloid impression material. Ruggeberg *et al.* have reported dimensional change and loss of reproduction of detail in Microstone® (Whip Mix Corporation, Louisville, KY, USA) casts using irreversible hydrocolloid immersed in a 0.5% sodium hypochlorite solution for ten minutes. Tullner *et al.* [15] have noted partial dissolution of irreversible hydrocolloid materials when immersed in a 1% sodium hypochlorite solution for 15 minutes [9]. The herbal solution we made is used as a spray. After spraying the surface of the impression material with herbal solution one has to wait for 1 to 2 minutes before pouring the impression. This will prevent any distortion of the impression surface as there is no

immersion of impression in the solution and time before pouring is also very less.

Prithi Jha, Asmitha and Latha studied the efficacy of an organic disinfectant ECOSAN R on alginate impressions. Component of Ecosan is Aloe Vera. They studied antibacterial properties of ecosan on alginate with swab technique. Result showed that use of ECOSAN R is good as compared to cleaning with water [10]. In our study we have used a polyherbal solution to get the antimicrobial benefits of neem, tulsi, aloe vera, gum acacia which gave solution better antimicrobial effect than cleaning with water.

De N, Ifeoma E in 2001 conducted study on antimicrobial effects of bark extracts of neem and concluded that there is a scientific basis for traditional use of extracts of bark and leaves of neem in skin infection. This study has proved scientifically that the component of bark of neem possesses significant antimicrobial activity against S aureus, S pyogen and P aeruginoa [11].

Study done by Amola Patil in the year 2018 he stated that antifungal activity of the Eugenol and linalool constituents of oil of Ocimum sanctum present in tulsi studied against two species of Candida (i.e. C. albicans and C. tropicalis) which commonly causes oral candidiasis and concluded that linalool is more promising and effective against candida [13]. Hence we have used tulsi extract in our herbal solution.

Hence in this study we have prepared an organic herbal disinfectant solution having multiple herbal ingredients with specific antimicrobial, cleaning, healing etc properties. Those ingredients there quantity and properties has been mentioned in the chart given below.

Ingredient, percentage and properties of ingredient used to prepare this herbal solution are as follow [14-19] these ingredients were calculated for 50µl/ml as at this concentration herbal solution was effective against the given bacteria's.

Table: Those ingredients there quantity and properties has been mentioned in the table

Ingredients used	%	Properties
Neem leave extract	0.108 gm	Antimicrobial, Antiviral, Antifungal
Tulsi leave extract	0.125 gm	Antimicrobial, Antiviral, Antifungal
Alove vera gel	0.40 gm	Antimicrobial, Antifungal, Soothing effect
Reetha extract	0.05 gm	Emulsifying and foaming action, Cleaning by reducing surface tension.
Ginger oil	0.1 ml	Antimicrobial, Antiviral, Antifungal
Neem oil	0.1ml	Antimicrobial, Antiviral, Antifungal
Peppermint oil	0.1 ml	Flavoring agent
Gum acacia	0.1 gm	Emulsifying agent, Stabilizer
Rock salt	0.10 gm	Preservative, Antimicrobial

That is why this solution not only boost effect of all natural ingredient against bacteria but also preserve the surface details of the impression. One more advantage is that the solution is cost effective.

Limitation of the study

The anti-microbial property was studid only for two microorganisms i.e. streptococcus mutans and candida albican as these are the most commonly found microorganisms in the oral cavity. Further studies can be done on variety of microorganisms found in oral cavity. The comparison of herbal disinfectant solution was done with water and not any commercially available disinfectant. Further studies can be carried out comparing the efficacy of commercially available disinfectants with herbal disinfectant on impressions.

5. Conclusion

These herbal ingredients have safest therapeutic benefits without any side effects on human body. Through this study herbal disinfectant solution can be considered as effective antimicrobial hence it can be used as a disinfectant agent and also prevent deleterious effect on alginate impression.

6. Financial support and sponsorship

Nil

7. Conflicts of interest

There are no conflicts of interest.

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