

# Effect of Tobacco Water on the Antibacterial Activity of Irreversible Hydrocolloid Impression Material

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**Abstract:** Infection control is a main concern for the dentist. This study aimed at determining the effect of tobacco water on the antibacterial activity of irreversible hydrocolloid impression material against four oral bacteria. The sample size consisted of 12 samples for each group. Three samples for determining antibacterial effect against each of the four oral bacteria. The antibacterial effect of four different concentrations of tobacco water mixed with irreversible hydrocolloid impression material on four oral bacteria. Diameters of zones of growth inhibition were measured. The results showed no zones of inhibition were obtained around the samples mixed with distilled water (control group). Zones of inhibition were seen around all the samples mixed with varying concentrations of tobacco water. This concluded that the diameters of zones of growth inhibition were increased as the concentrations of tobacco water mixed in irreversible hydrocolloid impression material increased.

**Keywords:** Irreversible hydrocolloid, tobacco water, antibacterial activity

## 1. Introduction

Infection control is an integral part of dental practice. Dental impressions harbor potential pathogenic bacteria due to their contact with the patient's saliva, plaque and blood, particularly irreversible hydrocolloid impression material.<sup>1</sup> Study of Powell and coworkers showed that 67 percent of impressions sent to dental laboratories showed contamination with bacteria such as *Enterobacter cloacae*, *Escherichia Coli* and *Klebsiella oxytoca*.<sup>2</sup> These impressions if not disinfected can cause cross infection between the dentists, dental assistants, staff, lab technicians and patient.<sup>1</sup> Based on the guidelines established by the American Dental Association (ADA) and Centers for Disease Control and Prevention (CDC), many methods of disinfection for irreversible hydrocolloid impression material have been proposed.<sup>3,4</sup> Among them, immersion and spray technique are the most commonly used techniques of disinfection.<sup>4</sup> However, in these techniques irreversible hydrocolloid impression material is susceptible to dimensional distortion. Also most of the disinfectants are irritants, and their vapour inhalation can cause health risks to the dental team.<sup>4</sup> Therefore, search for new methods and disinfection materials has led researchers to investigate the antibacterial compounds in plants. *Nicotiana plumbaginifolia* Viv. (Solanaceae) commonly known as 'Wild Tobacco' is an annual or perennial weed herb.<sup>5</sup> Tobacco nicotine inhibits the growth of pathogens which is dose dependent. It is equally affective against gram- positive and gram-negative bacteria, along with the acid-fast *Mycobacterium phlei* and the fungi *Candida albicans* and *Cryptococcus neoformans*.<sup>6</sup>

## 2. Materials and Methods

In this study, the irreversible hydrocolloid impression material (Zelgan 2002, Dentsply) was mixed with different

concentrations of tobacco water and its antibacterial effect was determined.

### Collection of plant materials and preparation of aqueous extract

Dried leaves of tobacco were grinded to fine powder using a grinder and stored in air tight bottle. Then different concentrations of tobacco powder were mixed with 250 ml of distilled water i.e. 2gm, 5gm, 10gm, 15gm in different conical flasks, air tight with cotton plugs and kept on a shaker for 8 hours. Then the extracts were filtered using a filter paper, and kept in airtight flasks at 4°C (Figure 1).

### Preparation of test and control specimens

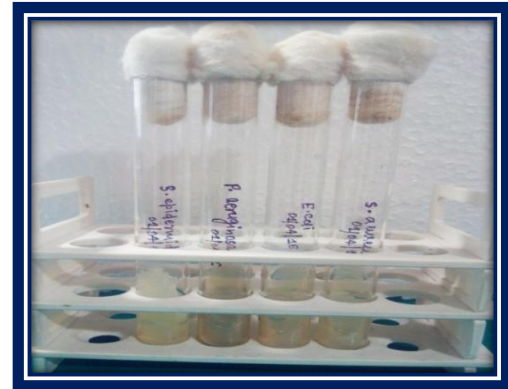
First, the irreversible hydrocolloid impression material was mixed according to the powder/liquid ratio (10 g/23 ml) recommended by the manufacturer. Sixty specimens were divided into five groups of 12 specimens each and were prepared accordingly,

- GROUP I (control group) : Specimens mixed with distilled water.
- GROUP II : Specimens mixed with 2 gm/250ml tobacco water.
- GROUP III : Specimens mixed with 5 gm/250ml tobacco water.
- GROUP IV : Specimens mixed with 10 gm/250ml tobacco water.
- GROUP V : Specimens mixed with 15 gm/250ml tobacco water.

Immediately after mixing, the material was placed in a mold and impression disks, 10 mm in diameter by 2 mm in thickness, were prepared (Figure 2). Mean weight of the disks was  $0.156 \pm 0.003273$  gm.



**Figure 1:** Different concentrations of tobacco water extracts kept in conical flasks



**Figure 3:** Four oral bacterias in test tubes



**Figure 2:** Sample discs prepared in a mold of 10mm in diameter by 2mm in thickness.



**Figure 4:** Specimens placed on the agar plates. For determining antibacterial effect.

### Measurement of antibacterial effect

The antibacterial effect was evaluated against four oral bacterias, namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Figure 3). Mueller Hinton Agar medium was prepared and poured into twelve plates. Three plates were prepared for each bacteria. These agar plates were then inoculated with the appropriate microorganisms under sterile conditions.

On each agar plate one specimen from each group was placed. Marking was done for the five groups, and specimens were selected from each of the five test groups (Group I, Group II, Group III, Group IV, Group V) and put onto the agar plates on their respective markings (Figure 4). The control specimen was placed in the centre of the agar plate. Three independent assays were performed for each microorganism ( $n = 3$ ). Finally, all plates were incubated for 24 to 48hrs at 37°C.

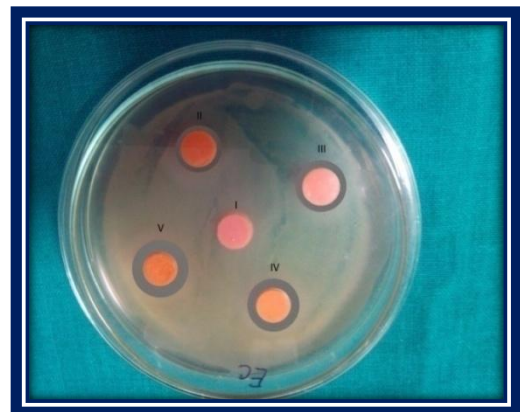
After incubation, the clear zones or inhibitory areas around the specimens were measured to evaluate the antibacterial effect.

### 3. Statistics

The results were reported and submitted to one-way ANOVA test ( $\alpha = 0.05$ ). One-way ANOVA and Tukey test analyses were performed using a statistical analysis program (SPSS 12.0, SPSS Inc, Chicago). Significance level was 0.05.

### 4. Results

Well defined areas of inhibited growth were seen after the incubation period (Figure 5). The results demonstrated that zones of growth inhibition around the specimens were observed on all plates. Mean diameters of zones of growth inhibition (mm) and standard deviation for each microorganism species and group are shown in table 1.



**Figure 5:** Zones of growth inhibition observed on agar plates

Species	Group I (Control)	Group II (2gm/250ml)	Group III (5gm/250ml)	Group IV (10gm/250ml)	Group V (15gm/250ml)
<i>Staphylococcus aureus</i>	0	13 ± 0.4	13.5 ± 0.2	14 ± 0.5	16 ± 0.3
<i>Staphylococcus epidermidis</i>	0	12.2 ± 0.4	13 ± 0.2	14.5 ± 0.4	15.8 ± 0.2
<i>Escherichia coli</i>	0	13.2 ± 0.6	14 ± 0.3	14.6 ± 0.5	16.2 ± 0.5
<i>Pseudomonas aeruginosa</i>	0	11 ± 0.5	11.6 ± 0.2	12 ± 0.4	13.3 ± 0.2

**Table 1.** Mean diameters of zones of growth inhibition (mm) and standard deviation for each microorganism species and group.

No zones of inhibited growth were observed around the control specimens on all agar plates. One- way analysis of variant and tukey test determined that the zones of inhibition tested became significantly larger as the concentration of tobacco in the aqueous solution was increased for all the four bacterias.

## 5. Discussion

Disinfecting dental impression is a must as microorganisms get incorporated in the impression material, as it remains in contact with the saliva and oral microbes, while setting. Alginate impression material disinfection techniques has several disadvantages, which led research to the developing of self disinfecting alginate material.

Disinfection of dental impressions is performed by using different disinfecting agents containing hypochlorite, glutaraldehyde and chlorhexidine. Effects of different concentrations of sodium hypochlorite on irreversible hydrocolloid impression materials were examined in different studies.<sup>7,8</sup>

Four representative microbes used in this study *S aureus*, *S epidermidis*, *E coli*, and *P aeruginosa* are pathogenic bacteria that have been widely used by other studies<sup>9,10,11</sup> as indicators of the effectiveness of disinfection protocols.

A large number of species of family *Solanaceae* growing mainly in the tropical and temperate region are rich in phytochemicals which have medicinal values. Some of these plants have great antibacterial activity against human pathogenic bacteria.<sup>12</sup>

Suffredini *et al.*, in 2004 tested aqueous extracts of *Solanaceae* members from native of Amazon rain forest and Atlantic forest for antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* following broth micro dilution method and they showed some degree of inhibition of bacterial growth at concentrations of 100 µ/ml.<sup>13</sup>

Nair *et al.*, in 2005 also reported that aqueous and ethanolic extracts from some plants used in allopathic medicine are potential sources of antiviral, antibacterial and antitumoral agents.<sup>14</sup>

Tobacco is a medicinal plant and it has anti-microbial properties. It is equally effective against gram positive and gram negative bacteria along with the acid fast mycobacterium *phlei* and fungi *candida albicans* and *Cryptococcus neoformans*.<sup>6</sup>

K.P. Singh et al in 2010 evaluated antibacterial activity of Aqueous and methanol extracts of *Nicotiana Plumbaginifolia Viv.* (Wild Tobacco) by paper disc diffusion method on five human pathogenic bacteria, namely *Bacillus cereus*, *Bacillus fusiformis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The results concluded that significant antibacterial effect were obtained by aqueous as well as methanolic extracts of leaves against all the tested bacteria.<sup>5</sup>

Alwahaab in 2012 conducted a study in which he investigated the effect of disinfecting solution (chlorhexidine digluconate) when incorporated in alginate powder instead of water against some microbes and on compressive strength of alginate, and the study concluded that the incorporation of disinfecting solution into impression material powder could serve an important role in infection control and chlorhexidine showed no adverse effect on the compressive strength of irreversible hydrocolloid impression material.<sup>15</sup>

## 6. Conclusion

Based on the findings of this study, we can conclude that ;

- Irreversible hydrocolloid impression material mixed with aqueous solution of tobacco water can exhibit varying degrees of antibacterial activity.
- The recommended concentration of aqueous solution of tobacco water to produce antibacterial activity in irreversible hydrocolloid impression material is 15gm/250ml.

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## Author Profile



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