JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH, 2020
VOL 11, NO. 5

RESEARCH ARTICLE

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# Effect of Ethanolic Extract of *Citrullus lanatus* (Watermelon) Against Oral Microflora in Orthodontic patients

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**ABSTRACT**

**Aim:** Streptococcus mutans and Lactobacillus acidophilus is one of the most common oral microflorae responsible for the formation and progression of dental caries respectively. Patients undergoing orthodontic treatment have alterations in their micro flora due to increased accumulation of debris around orthodontic auxiliaries. With our previous study we have found that there was significant antibiotic effect for watermelon juice against lactobacillus. The aim of the current study is to find the effect of ethanolic extract of *Citrullus lanatus* (watermelon) against oral microflora such as Lactobacillus acidophilus and Streptococcus mutans in patients undergoing orthodontic treatment.

**Materials and methodology:** Ethanolic extract of watermelon was prepared. Three different concentrations of the extract were used to find its antibiotic effect against oral microflora such as lactobacillus and streptococcus mutans. Paper discs incorporated with the extract were incubated in freshly prepared colonies of lactobacillus acidophilus and streptococcus mutans. After incubation period the culture plates were examined for antibiotic sensitivity.

**Results:** Results showed that there was no decrease in the bacterial colony count in the culture plate containing three different concentrations of ethanolic extract of watermelon.

**Conclusion:** Though watermelon juice had significant effect against lactobacillus, the Ethanolic extract of *Citrullus lanatus* had no significant antibacterial effect against lactobacillus acidophilus and streptococcus mutans in patients undergoing orthodontic treatment.

**ARTICLE HISTORY**

Received October 20, 2020
Accepted November 22, 2020
Published December 24, 2020

**KEYWORDS**

Watermelon, Lactobacillus, Streptococcus mutans, Oral flora, Oral hygiene, Malocclusion, Orthodontics.

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## INTRODUCTION

Patient undergoing orthodontic treatment are more prone for alteration in the oral microbiological profile. This is due to the increased accumulation of plaque and food debris in various orthodontic auxiliaries such as brackets, bands, wires, etc., additional oral hygiene aids are essential to maintain the patient's oral hygiene. Due to its long-term side effects prescribing a patient with chlorhexidine mouth wash for the entire course of the orthodontic treatment is not advisable. So, it is essential to find an alternative for the same to reduce oral microflora. With our earlier studies we have found out the antibacterial property of watermelon juice [1]. In this

Malocclusion is one of the most common dental conditions that we come across in our day to day practice. Facial aesthetics plays a crucial rule in social and psychological development of an individual [2]. Patients with malocclusion are more prone for inflammatory conditions such as gingivitis and periodontitis. This is because the patients find it difficult to manage their oral hygiene as the tooth surface becomes less self-cleansing which will lead to increased accumulation of plaque and calculus.

Due to increased aesthetic demands the number of people who are undergoing orthodontic treatment has increased in the past decade. People who were not satisfied with their appearance were one of the most common who seek orthodontic treatment [3]. The use of fixed orthodontic appliance alters the oral microbiological profile of the patient. Patients undergoing orthodontic treatment are more prone for increase in the level of bacteria such as streptococcus mutans or lactobacillus. Lactobacillus are gram-positive, facultative anaerobic, round bacteria and Streptococcus mutans are one of the most common bacteria seen in oral environment and they belong to the Streptococcaceae family.

Though there are various oral hygiene measure available such as Tooth brushes, Interdental brushes, automated brushes, Dental floss, Mouth washes etc., patients find it difficult to manage their oral hygiene. This is due to the presence of various auxiliaries of fixed orthodontic appliances such as the brackets, wires, bands, elastomeric rings etc., so it is very essential to provide proper oral hygiene instructions to all the patients undergoing orthodontic treatment, along with other post-bonding instructions. The patient should follow all these strict oral hygiene protocol to maintain proper oral hygiene and prevent bacterial infections which occur from accumulated plaque or calculus.

The use of herbal products and home remedies for oral hygiene maintenance has been started becoming prevalent in the past few decades. Antimicrobial effect of fruit juices was studied earlier [4]. The aim of this study is to evaluate the antibacterial property ethanolic extract of *Citrullus lanatus* watermelon against oral microflora such as Lactobacillus acidophilus and Streptococcus mutans in patients undergoing orthodontic treatment.

## MATERIALS AND METHODOLOGY

Ethanolic extract of *Citrullus lanatus* was obtained from fresh cut watermelon which was obtained in a local market. Thin slices of watermelon were cut and sundried. 10 gm of the sundried watermelon was taken in a conical flask and ethanol was added to it till the sundried pieces were completely submerged [Figure 1]. The conical flask was stirred every day and at the end of third day the extract was collected in test tubes.

The ethanol was allowed to evaporate till we obtained a concentrated extract of watermelon. Furthermore, the dried pieces of watermelon after collection of the extract and evaporation of the remaining ethanol was grinded using mortar and pestle. Few millilitres of the collected extract were added to the same so that highest concentration of the extract can be obtained.

The ethanolic extract watermelon obtained was autoclaved to obtain a sterile solution and was used for the study [Figure 2]. Freshly obtained colonies of lactobacillus and streptococcus mutans were used for the study. The colonies were incubated in culture plates containing lactobacillus agar and nutrient agar respectively. These culture plates were for the antibiotic sensitivity test.

The ethanolic extract of watermelon is incorporated in paper disks in three different concentrations and was used for doing the antibiotic sensitivity test. Finnpipette F3 device was used to micropipette three different concentrations of the extract (Figure 3). The three different concentrations were as follows,

100 percent concentration containing 20 micro litres of the extract.

75 percent concentration containing 15 micro litres of the extract and 5 micro litres of distilled water.

50 percent concentration containing 10 micro litres of the extract and 10 micro litres of distilled water

The disks were incorporated with the extract (Figure 4,5) and were placed in the culture plates containing the cultures of freshly prepared lactobacillus and streptococcus mutans colonies made from samples obtained from patients undergoing orthodontic treatment. The samples were collected using sterile cotton swabs from the tooth surface around the brackets. Five disks were placed in each culture plate and were incubated for 24 hours.

## RESULTS

The incubated culture plates containing the freshly prepared colonies of lactobacillus and streptococcus mutans and disks with three different concentrations of ethanolic extract of watermelon were examined for the colony counts and antibiotic

sensitivity by disk diffusion method. The test antibiotic begins to diffuse outward from the disks, creating a gradient of extract concentration in the agar such that the highest concentration is found close to the disk and it decreases further away from the disk.

The size of this zone depends on various factors, one being the effectiveness of the antibiotic at stopping the growth of the bacterium and another factor is that will influence the size of a zone was the diffusion of the antibiotic within the agar medium and varies based on the molecular configuration of the antibiotic. The zone diameter was measured and was compared to a database of zone standards to determine if the bacterium studied was susceptible, moderately susceptible or resistant to the ethanolic extract of watermelon.

There was no zone of inhibition around the disks (Table 1). It was found that there was no antibiotic effect for the ethanolic extract of watermelon against oral microflora such as lactobacillus acidophilus and streptococcus mutans. This shows that the bacterium was resistant to the ethanolic extract of watermelon. The culture plates showing the colonies of lactobacillus and streptococcus mutans with the antibiotic test disks are shown in the pictures below (Figure 6,7,8).

## DISCUSSION

In our previous study we have found out that the antibacterial effect of watermelon juice against oral microflora. Watermelon juice had significant antibacterial effect against lactobacillus acidophilus. In the current study we studied the antibacterial effect of ethanolic extract of watermelon against Lactobacillus acidophilus and Streptococcus mutans and found out that there is no effect.

Patients undergoing orthodontic treatment are more prone of alterations in their oral microbiological profile. This is due to the presence orthodontic attachments and auxiliaries on the tooth surfaces of patients undergoing orthodontic treatment. This will lead to increased accumulation of plaque and calculus and difficulty in oral hygiene maintenance. There are many studies which showed the alterations in the oral microflora in the patients undergoing fixed orthodontic treatment. Edith Lara-Carrillo et al [5] and Sepideh arab et al [6] studied the effect of orthodontic treatment on saliva, plaque and also the levels of microbial colony count in the oral cavity. Their study showed that there were major changes in the salivary properties such as increase in the stimulated flow rate, the buffer capacity and salivary pH, which augment the anti caries activity of saliva. But, increased in the occult blood indicated there was more gingival inflammation, which was augmented by the retention plaque on the tooth surfaces and the difficulty in maintaining a good oral hygiene. They studied both the microbiological and the non-microbiological parameters of saliva and stated that the presence of attachments lead to increased accumulation of plaque and thus resulted in alterations in the microbiological profile in the oral cavity of the patient.

Amanda Osório Ayres de Freitas et al [7] did a systematic review on the effect of orthodontic treatment on oral microflora and showed that there was only moderate evidence for the alterations of the quality and quantity of oral microorganisms by orthodontic treatment. They reviewed the literature from various articles which studied the alterations in the bacterial count in the oral cavity after fixed orthodontic treatment. They took 8 articles according to their inclusion criteria and took only 4 articles with moderate methodological quality into the review. Anyways further study with recent research should be included to make the results of the review more reliable. A comprehensive study on the effect of orthodontic treatment on oral health status was studied by Kenan Cantekin et al [8] showed that DMFT counts and the PI increased in a group of young dental patients who underwent orthodontic therapy, and thus patients undergoing orthodontic treatment should follow very rigid oral hygiene protocols. Proper oral hygiene instructions should be given to the patients and the same should be reflected in the patient's oral hygiene which should be monitored during every appointment of the patient. Delphine MARET et al [9] and Kristina Peros et al [10] studied the effect of orthodontic treatment on salivary non-microbial and microbial parameters. The studies showed that there was significant increase in the lactobacillus acidophillus and streptococcus mutans bacterial colony counts.

There were various studies which were carried out for studying the reduction of microbial colony count using dietary alterations and herbal products. The reduction of lactobacillus acidophilus by periodic restriction of carbohydrates was studied by Philip jay et al [11] showed that though there was no significant reduction in the lactobacillus count and there was significant reduction in caries activity. Sule Kavaloglu Cildir et al [12] conducted a study on the amount of reduction of streptococcus mutans bacteria in saliva in patients undergoing fixed orthodontic treatment by daily consumption of yoghurt containing probiotic bacteria and stated that short term consumption of fruit yoghurt everyday containing Bifidobacterium animalis may reduce the levels of mutans streptococci in saliva. The Antibacterial activity of Acacia catechu willd bark against Streptococcus sanguis, Streptococcus mitis and Lactobacillus acidophilus was studied by Lakshmi T et al [13] and stated that these extracts had bactericidal action against lactobacillus and it was due to the phytochemicals present in it. The effect of Traditional Medicinal Plant Extracts and Natural Products against Oral Bacteria was reviewed by Enzo A. Palombo [14] and showed that the plant

extracts or phytochemicals that inhibit the growth of oral pathogens will reduce the development of plaque accumulation on the surfaces of the dentition, influence the adhesion of bacteria to surfaces and reduce the symptoms of diseases of oral cavity. The Antibacterial Activity of Ethanolic root extract of Glycyrrhiza glabra against oral bacteria was studied by Geetha RV et al [15] and they stated that the extract at different concentration had antibacterial activity against oral bacteria such as Lactobacillus acidophilus, and various Streptococcus species.

Though there were many oral hygiene measures which available in the market we decided to create a new adjunct to the same by the use of watermelon juice and studying its antibacterial effect. Antibacterial activity of fruits against Escherichia coli was studied by Misbah Manzoor et al [16] and stated that extract of apricot had the maximum inhibition effect followed by the extract of watermelon. The various medicinal uses of watermelon were reviewd by EO. Erhirhie et al [17] and showed that Citrullus lanatus possesses numerous bioactivities from the natural sources which is of better advantage than the conventional therapies. The antibacterial activity of watermelon seed was studied by Adelani-Akande Tabitha Adunola et al [18] and they stated that The antibacterial effects of C. lanatus [watermelon] seed extracts against the selected bacteria suggests that the extracts obtained by different extraction methods such as the cold maceration, Soxhlet extraction, as well as using methanol and chloroform methods had potential as antibacterial agents especially against Staphylococcus species and P. aeruginosa.

The significant antibacterial effect of watermelon juice against lactobacillus and no effect of ethanolic extract of *Citrullus lanatus* against lactobacillus and streptococcus mutans shows that the antibacterial effect of watermelon is present in the seeds, the antibacterial activity of which has been studied earlier.

## CONCLUSION

From this study it could be concluded that though watermelon juice had significant antibacterial effect against lactobacillus, the ethanolic extract of *Citrullus lanatus* had no effect against lactobacillus acidophillus and Streptococcus mutans. Thus showing that antibacterial effect of watermelon is present significantly in the seeds. But since this is an invitro study and has very minimal evidence, future studies on patients and its effect against other bacterias are needed to further validate this study and the earlier studies.

**Declaration of Conflicting Interests Policy**

The Authors declares that there is no conflict of interest

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**Table 1: Effect of ethanolic extract of watermelon against lactobacillus acidophilus and streptococcus mutans**

| Concentration of the watermelon extract / Bacteria tested | 50% | 75% | 100% |
|---|---|---|---|
| Lactobacillus acidophilus | No zone of inhibition (Resistant) | No zone of inhibition (Resistant) | No zone of inhibition (Resistant) |
| Streptococcus mutans | No zone of inhibition (Resistant) | No zone of inhibition (Resistant) | No zone of inhibition (Resistant) |

Resistant: No antibiotic effect against the bacterium


**Figure 1: Sun dried watermelon immersed in ethanol**

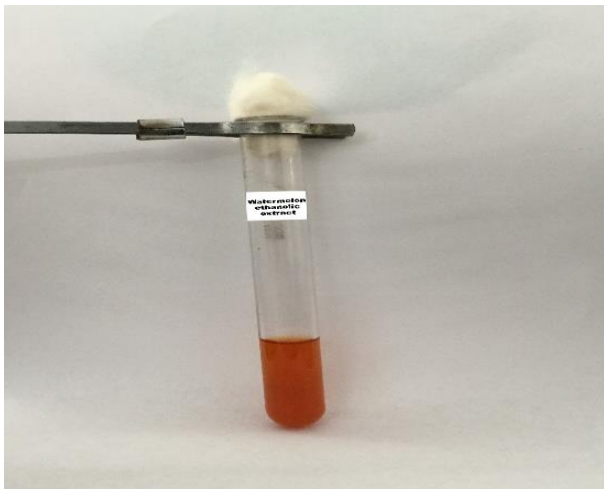

**Figure 2: Ethanolic extract of watermelon**


**Figure 3: Finnpipette f3 used for pipetting**

**Figure 4: Extract pipetted out to the paper disks**

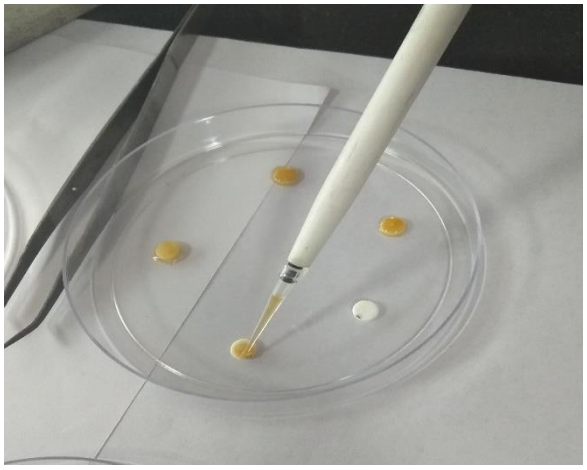

**Figure 5: Extract pipetted out to the paper disks**

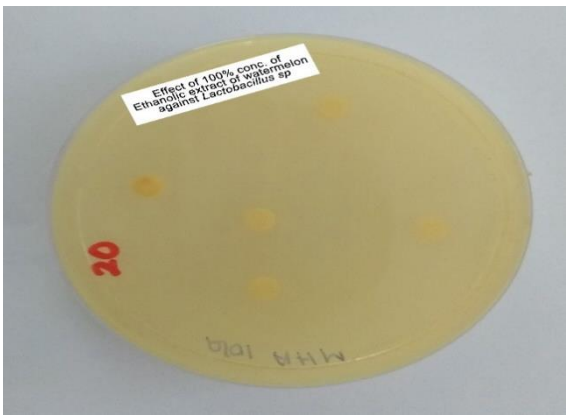


**Figure 6: Negative result for 100% concentration of the extract**

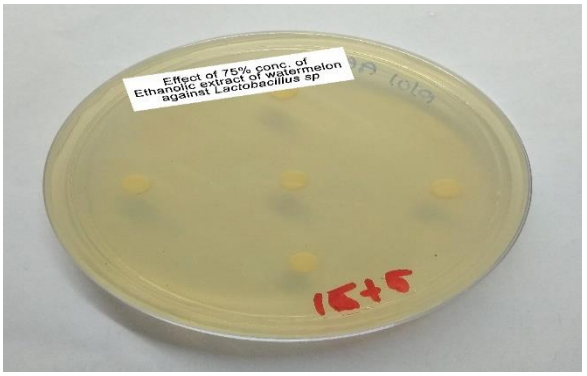


**Figure 7: Negative result for 75% concentration of the extract**

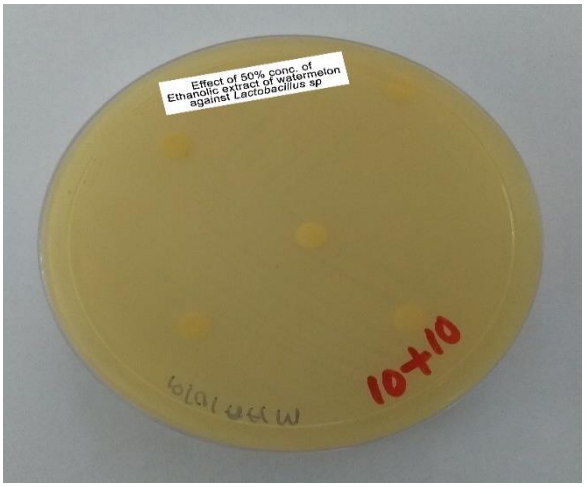


**Figure 8: Negative result for 50% concentration of the extract**