

# Antibacterial Activity of Guava Leaves Extract Against *Lactobacillus Acidophilus*: An In-Vitro Study

Priya Gurnani<sup>1</sup>, Ajith Krishnan C.G<sup>2</sup>, Rajendra Gurnani<sup>3</sup>, Abhishek Ghosh<sup>4</sup>, Ankit Shah<sup>5</sup>

1,4,5- Post graduate student, Department of Public Health Dentistry, K.M Shah Dental College & Hospital. 2- Professor and HOD, Department of Public Health Dentistry, K.M Shah Dental College & Hospital. 3- General Physician, Satguru Hospital, Sardar Patel Nagar, Near Airport, Ahmedabad.

Correspondence to:  
Dr. Priya Gurnani, Post graduate student, Department of Public Health Dentistry, K.M Shah Dental College & Hospital.  
Contact Us: www.ijohmr.com

## ABSTRACT

**Introduction:** Substances used for preventing caries has got many side effects. Hence experiments should be conducted on naturally available herbs and plants to explore their antibacterial and anticariogenic effect. **Objectives:** To assess the effect of 5%, 10%, 15% and 20% concentration of ethanol, DMSO (Dimethyl sulphoxide), and water extracts of guava leaves against *Lactobacillus acidophilus*. **Materials and Methods:** An invitro study was conducted in laboratory of microbiology. Extracts of guava leaves with ethanol, water & DMSO were prepared by using Soxhlet extractor. Four concentrations 5%, 10%, 15% and 20% weight/volume of ethanol, water & DMSO extracts were prepared. Agar well-diffusion method was employed to test the antibacterial efficacy. 8 plates each were prepared for the three extracts. Chlorhexidine (0.2) & distilled water were used as positive & negative control. **Results:** Only two extracts i.e. ethanol & water of *P. guajava* & 0.2% chlorhexidine showed activity against both *L. acidophilus*. Maximum zone of inhibition was observed with CHX. Mean zone of inhibition produced by 0.2% chlorhexidine was 22.25mm & by 20%, 15%, 10% and 5% ethanolic extract was 21.34mm, 17.56mm, 16.14mm and 15.34mm respectively, and least activity was shown by water extract & no zone of inhibition was observed with DMSO extract. 20% ethanolic extract of guava was found as efficacious as 0.2% chlorhexidine. **Conclusion:** Only the extracts prepared with ethanol and water were found to have antibacterial effect against *L. acidophilus* and 20% ethanolic extract of guava was found as efficacious as 0.2% chlorhexidine.

**KEYWORDS:** Guava Leaves, *Lactobacillus*, Dental Caries

## INTRODUCTION

Dental caries is a global oral health problem.<sup>1</sup> It poses a major burden in the form pain, discomfort and affecting the quality of life. It is the dentists who carries the responsibility to prevent it.<sup>2</sup> The main etiologic factor involved is the presence of bacteria which causes acid formation and bring the plaque pH down i.e. critical pH.<sup>3</sup> The microorganism responsible for the initiation of dental caries is *Lactobacillus acidophilus*.<sup>5,6</sup> So any chemical substance which can act against *Lactobacillus* can help in reducing the occurrence of caries. The antimicrobials penicillin, ampicillin, tetracycline, erythromycin and vancomycin have efficacy to prevent dental caries in vitro & in vivo. But these drugs have many side effects such as nausea, bacterial resistance, diarrhoea etc.<sup>7</sup> Chlorhexidine is considered to be the gold standard for the same.<sup>8,9</sup> But it has numerous side-effects such as staining, alteration of taste sensation, parotid duct stenosis.<sup>10-12</sup> Therefore it is necessary to explore naturally available products which can act against the caries causing bacteria thus helping out in preventing the latter and also is safe.<sup>13</sup>

*Psidium guajava* is a phytotherapeutic plant commonly

known as Guava.<sup>14</sup> The stem and leaves of the guava plant contains many antioxidants which have many activities beneficial for whole human body.<sup>14</sup> It acts as a medicine in a number of diseases such as skin diseases, sore throat, dysmenorrhoea, dysentery.<sup>15</sup>

Studies have proved that guava leaves can be very useful in maintaining oral hygiene.<sup>16</sup> The pharmacological activities of guava is due to its contents such as tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibres and fatty acids. Mainly the antibacterial activity is due to the flavonoid called quercetin.<sup>17</sup>

Studies conducted in the past focused on the efficacy of other parts of the plant against periodontal diseases and oral malodor and some have focused on its effect in preventing the formation of dental plaque which is thought to contain the bacteria responsible for initiation and progression of dental caries.

Hence the present study was conducted to assess the antibacterial efficacy of ethanolic, Dimethyl sulfoxide

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(DMSO) and water extracts of Guava leaves at 5%, 10%, 15% and 20% weight/volume (w/v) concentrations against *L. acidophilus*.

## MATERIALS & METHOD

**Study Design:** Experimental in-vitro study

**Study Setting:** The study was conducted in the laboratory of Microbiology, Ahmedabad. The ethical clearance had been obtained from the Ethical review committee of Sumandeep Vidyapeeth, Vadodara.

**Test Organisms:** Test organism was *L. acidophilus*, which was bought from MTCC gene bank, Institute of Microbial Technology (MTCC No. 10307), Chandigarh, India. *L. acidophilus* was obtained in lyophilized form in a glass vial. The revival of bacteria was done using nutrient broth having a pH of 7.4. The nutrient broth containing bacteria was kept in an incubator at 37°C for 48 hours.

**Preparation of extract:** Four concentrations, i.e., 5%, 10%, 15% and 20% w/v were tested for antibacterial activity. The guava leaves were collected from a household garden. Leaves of *P. guajava* L. (Myrteceae) were cleaned and dried in an oven at 60°C for 5 hours. The dried leaves were then grounded to powdered form. Extract was prepared using soxhlet extractor. The extracts were filtered using Whatman no. 4 filter paper and then dried in a rotary evaporator for 5-6 hours at 60°C. The required concentrations of 5%, 10%, 15% and 20% ethanolic extract were prepared by adding 0.05g, 0.1g, 0.15g and 0.2g of powder respectively in 10 ml of ethanol. Similarly for the water extract the same amount was added in the distilled water. The extracts were stored at 4°C in sterile bottles.

**Antibacterial Test:** The antibacterial activity of guava extracts was checked by agar well - diffusion method which was performed on the next day of preparation of the extract. A measured amount, that is, 0.5 ml of suspension of inoculums having  $3 \times 10^8$  *L. acidophilus*/ml (estimated using Mc Farland standard) was streaked on de Man, Rogosa, Sharpe (MRS) agar. Three groups of plates were prepared: Ethanolic extract group, DMSO (Dimethyl sulfoxide) extract group and water extract group.

In each group, there were 8 plates. In all the plates, 4 wells were punctured in agar with the help of well borer. 4 wells prepared in ethanolic extract group were filled carefully with 0.08 ml 5%, 10% ethanolic extract of guava, 0.2% chlorhexidine (positive control) & sterile distilled water (negative control) in 4 plates & 15%, 20% guava leaves extract, 0.2% chlorhexidine (positive control) and sterile distilled water (negative control) in other 4 plates. Similarly in DMSO and water extract groups. All the plates were kept in an incubator at 37°C for 48 hours. After 48 hours zones of inhibition were measured.

**Statistical analysis:** Statistical analysis was performed using Statistical Package for the Social Sciences 17.

ANOVA test was applied to know the difference between the groups such as 5%, 10%, 15% & 20% ethanolic, DMSO extract and 0.2% chlorhexidine and 5%, 10%, 15% & 20% water extract and 0.2% chlorhexidine. *Post-hoc* analysis was employed to specifically find that between which groups did significant difference existed.  $P < 0.05$  was considered as statistically significant.

## RESULTS

The present study was conducted to assess the efficacy of Guava leaves extracts on *L. acidophilus* using agar well-diffusion method. Mean zone of inhibition exhibited by 20%, 15%, 10% and 5% ethanolic extract was 21.34mm, 17.56mm, 16.14mm and 15.34mm [Table 1]. Water extract at similar concentrations exhibited 8.73mm, 7.28mm, 3.59mm and 3.31mm zones of inhibition [Table 1]. No zone of inhibition was seen with Dimethyl sulfoxide extract at the similar concentrations. Highest mean zone of inhibition was exhibited by 0.2% chlorhexidine (22.25 mm) [Table 1].

Group		N	Mean Zone of Inhibition	Std. Deviation
Ethanol	5%	8	15.34	0.103
	10%	8	16.14	0.100
	15%	8	17.56	0.199
	20%	8	21.34	0.164
Water	5%	8	3.31	0.088
	10%	8	3.59	0.127
	15%	8	7.28	0.090
	20%	8	8.73	0.119
DMSO	5%	8	0.00	0.000
	10%	8	0.00	0.000
	15%	8	0.00	0.000
	20%	8	0.00	0.000
CHX	0.2%	8	22.25	21.00
Distilled Water	-	8	0.00	0.00

Table 1 - Mean inhibition zones of varying concentrations of Ethanolic, DMSO and water extracts of guava leaves, Chlorhexidine & Distilled Water

The antibacterial efficacy of ethanolic extract of guava leaves at 5%, 10%, 15% & 20% concentration and 0.2% chlorhexidine was compared using one-way ANOVA ( $P < 0.05$ ) [Table 2]. Results that showed a significant difference were further analyzed for statistical significance between specific groups using Tukey post-hoc analysis. There was a significant difference between the efficacy of 5%, 10%, 15% and 20% ethanolic extract and 0.2% chlorhexidine. On post-hoc analysis, it was revealed that the efficacy of 20% ethanolic extract (21.34 mm) was not significantly different than 0.2% chlorhexidine (22.25 mm) ( $p = 0.271$ ). However, the efficacy of 5%, 10% & 15% ethanolic extract was significantly lower than 0.2% chlorhexidine ( $P = 0.012$ ).

Concentrations	N	Mean zone of inhibition (mm)	Std. Deviation	
Ethanolic Extract of Guava				
	5%	8	15.34	0.29
	10%	8	15.98	0.28
	15%	8	17.56	0.56
	20%	8	21.34	0.46
CHX				
0.2%	8	22.25	0.76	

\* $P=0.000$ ,  $P<0.05$  considered statistically significant

Table 2- Comparison of antibacterial efficacy (inhibition zone) of 5%, 10%, 15% and 20% ethanolic extract of guava leaves against *L. acidophilus*

There was a significant difference in the activity of all the four concentrations of water extract and 0.2% chlorhexidine ( $P < 0.05$ ) [Table 3]. *Post-hoc* analysis revealed that the activity of 0.2% chlorhexidine was significantly higher than 5%, 10%, 15% water extract as well as 20% water extract of guava leaves. The effect of water and ethanolic extract of guava were found to be dependent on the concentration. 20% concentration of water and ethanolic extracts was significantly higher than 15% concentration. Further 15% concentration had higher zone of inhibition than 5% and 10%.

Concentrations	N	Mean zone of inhibition (mm)	Std. Deviation
Water Extract of Guava			
5%	8	3.31	0.25
10%	8	3.59	0.36
15%	8	7.28	0.25
20%	8	8.73	0.34
CHX			
0.2%	8	22.25	0.76

\* $P = 0.000$ ,  $P < 0.05$  considered statistically significant

Table 3 - Comparison of antibacterial efficacy (inhibition zone) of 5%, 10%, 15% and 20% water extract of guava leaves against *L. acidophilus*

## DISCUSSION

In the present study extracts of guava leaves were tested against *L. acidophilus*. The guava leaves had been reported to contain essential oils, flavonoids, saponins, nerolidiol,  $\beta$ -sitosterol, ursolic, crategolic and guayavolic acid. These substances were reported to have strong antibacterial action.<sup>18-22</sup> Prabu *et al.*, have demonstrated Guajaverin, a flavonoid in the methanolic extract of leaves of guava that exhibited strong antibacterial activity against caries causing *Streptococcus mutans*.<sup>23</sup> Thus, this study attempted to evaluate the efficacy of guava leaves, a rich source of antibacterial substances against caries causing *L. acidophilus*.

In this study, agar well-diffusion method was employed for microbiological assay that had been reported to be more sensitive than other methods like disc diffusion method.<sup>24</sup> *L. acidophilus* was cultured on MRS agar as per the recommendations of MTCC Chandigarh.

Apart from leaves, extracts from other parts of guava had also been found to possess antibacterial activity. Ngoroyemoto *et al.*, studied the ethanolic and methanolic extracts from roots of guava and found these to be effective against *L. acidophilus*.<sup>25</sup>

According to table 1 the efficacy of 5%, 10%, 15% and 20% ethanolic extract was found to be better than the water extract at the similar concentrations. This is because the water and ethanolic extracts differ in their composition. Ethanolic extract contains tannins as well as flavonoids, whereas water extract contains tannins but not flavonoids. This difference in composition of ethanolic and water extract can be attributed to the difference in solubility of various components of guava leaves in water and organic solvents.<sup>26</sup> Flavonoids had been reported to exhibit good antibacterial activity.<sup>27</sup>

Similarly, the difference in the activity of different concentrations of the water extract of guava leaves against *L. acidophilus* can be attributed to the difference in the concentration of

different antibacterial compounds present in the 5%, 10%, 15% & 20% concentrations of water extract.

In the table 2 & 3, the antibacterial efficacy of ethanolic & water extract of guava leaves at 5%, 10%, 15% & 20% concentration and 0.2% chlorhexidine was compared using one-way ANOVA. It was observed that the efficacy of 20% ethanolic extract is comparable to 0.2% chlorhexidine. So the results of the present study demonstrated almost similar efficacy of 20% ethanolic extract of guava leaves and 0.2% chlorhexidine. But the efficacy of the all the four concentrations of water extract was found to be lesser than the ethanolic extract as well as 0.2% chlorhexidine.

However, no zone of inhibition was observed with the DMSO (dimethyl sulfoxide) extract at 5%, 10%, 15% and 20% concentrations. DMSO is known to be an inert solvent which is an organosulfur compound with the formula  $(\text{CH}_3)_2\text{SO}$ . This colorless liquid is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water<sup>28</sup>. This was used in the present study to see whether the antibacterial activity of guava is due to itself or because of the components of solvents such as alcohol, water, etc. As no zone of inhibition was seen, it is assumed the extract of guava leaves acts as an antibacterial agent in synergy with the components of the solvent.

The present study evaluated qualitatively the antimicrobial potential of guava leaves extract against *L. acidophilus*. However, further quantitative research is needed to know the minimum inhibitory concentration and to evaluate the effectiveness and safety of guava extracts *in vivo*.

## CONCLUSION

Ethanolic and water extracts of guava leaves possess antibacterial activity against *L. acidophilus* with 20% ethanolic extract being as efficacious as 0.2% chlorhexidine. Natural products such as guava leaves having antibacterial activity may be used as economical and suitable adjuvant to synthetic medicines and compounds and their judicious use might not only help to inhibit the side effects of synthetic chemicals but also prove to be cost effective in developing economies

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