

## Evaluation of Antibacterial Efficacy of Terminalia Chebula Fruit Extracts against Enterococcus Faecalis

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### Abstract

The usage of plant based therapeutics in medicine and dentistry is increasing because of their natural origin with higher safety margins and lesser or no side effects. *Terminalia chebula* (Haritki/ Harad) also known as “king of medicine” with established effects against various bacterial and fungal infections including the dental caries pathogens was tested in this study against *Enterococcus faecalis*, which is one of the persistent pathogen found in root canal post treatment disease.

**Aim:** The aim of this study was to evaluate the possible antibacterial efficacy of T. chebula fruit extracts (aqueous and ethanolic) against E. faecalis and its comparison to that of 2% chlorhexidine.

**Methodology:** For this purpose both aqueous and ethanolic extracts of the fruit were used against the bacteria using the Agar well diffusion method and the zones of inhibition surrounding the wells indicating the antibacterial activity were recorded.

**Results:** In the present study the results were found promising as the fruit extracts tested exhibited zones of inhibition against the tested bacteria. Higher activity was shown by ethanolic extract than an aqueous extract of the fruit. However both the extracts exhibited less activity as compared to 2% chlorhexidine. The results were statistically analysed.

**Conclusion:** Within the limitations of the study the promising findings suggest the presence of antibacterial activity in the tested plant material against *E. faecalis*. The findings are suggestive of potential which further needs extensive research.

**Key words:** Antibacterial, E. faecalis, Herbal extract, T. chebula

### Introduction

Medicinal plants are part and parcel of human society from the dawn of civilization to combat diseases and have been considered valuable and cheap source of unique phytoconstituents which are used extensively in development of drugs against various diseases<sup>1,2,3</sup>. Terminalia Chebula is flowering evergreen tree native to India, Pakistan, Nepal, Srilanka and South west of China. It is acclaimed as the “King of Medicine” in Tibet. Herbal drugs and remedies have long history of use for gum and tooth problems and antibacterial activities of T. Chebula extracts against several bacteria have been reported<sup>4</sup>. T. chebula has been found to be an active component of Triphala (a natural agent which has been listed as a root canal irrigant)<sup>5,6</sup>. In the past few years Enterococcus faecalis has been the focus of increased interest both in medicine and dentistry. A recognized pathogen in post treatment endodontic infection, eradication from root canal with chemomechanical preparation and using disinfecting irrigants and antibacterial dressings is difficult<sup>7</sup>.

The present in vitro study focuses on the antibacterial activity of T. chebula, ethanolic and aqueous extracts against bacteria E. Faecalis and is compared with medicament 2% Chlorhexidine.

### Materials and Methods

**Collection of Sample Fruit:** Fruits of T. chebula tree were obtained, shade dried and a powder was made. The

powder was then used to prepare two types of extracts 10% aqueous and 10% ethanolic with the help of shaking incubator and fractional distillation method.

**BHI agar:** Brain heart infusion agar was prepared according to manufacturer’s instructions for petri dishes and autoclaved at 121°C at 15 psi for 15-20 minutes. BHI broth was also prepared and stored in 5ml vials for 2 days.

**Isolation of Enterococcus Faecalis:** Cultures of E. faecalis were grown overnight in BHI broth at 37°C and bacterial growth was checked by changes in turbidity after 24 hours.

**Inoculation with Enterococcus Faecalis:** BHI broth which was inoculated with E. faecalis was used to inoculate 15 BHI agar plates, by pour plate method. After 24 hours of incubation, uniform lawn of E. faecalis appeared on BHI agar plates. Agar well diffusion method was used to derive results.

**Placement of Medicaments:** On marked petri dishes total four wells of 8mm diameter were prepared with the help of metal borer. In these prepared wells, material was placed with a micropipette. Out of four wells, one was filled with distilled water followed by 2% chlorhexidine. Terminalia. Chebula aqueous and ethanolic extracts respectively. After the placement of various medicaments in the prepared walls, these plates were again incubated for 24 hours at 37° C

**Zones of Inhibition:** The next step was to measure the diameter of zones of inhibition that appeared around

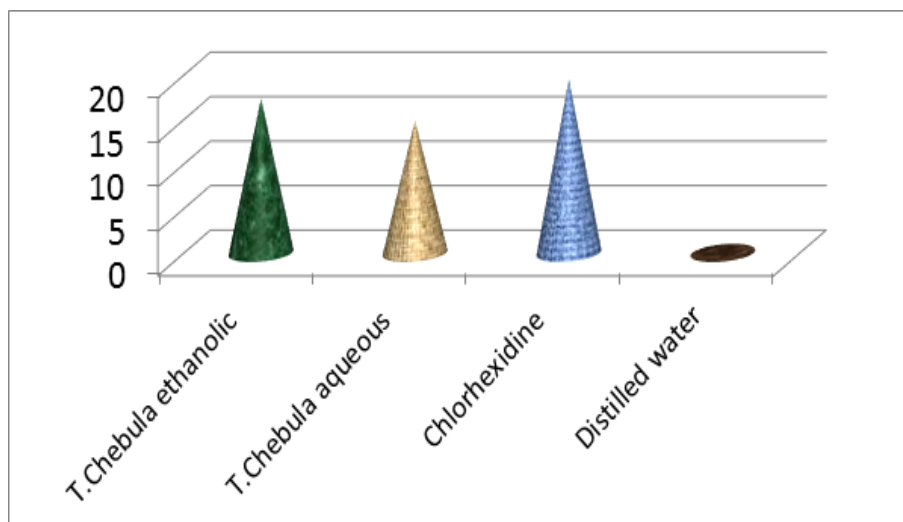
each wall. Keeping the lid in place a ruler was used to measure the diameter of clear area in millimetres. All manipulations were performed under laminar air flow to avoid contamination.

### Observations and Results

The decreasing order of antibacterial as observed by measuring the zones of inhibition and analysing the values obtained (Table 1) with One way ANOVA, turkey multiple comparison means (as depicted in Table 2 and Graph 1) was: Chlorhexidine > Terminalia chebula ethanolic > T.chebula aqueous > Distilled water

S. No.	Control (n=15)	T.Chebula Ethanolic (n=15)	Chlor-hexidine (n=15)	T.Chebula aqueous (n=15)
1.	0	22	18	13
2.	0	15	21	16
3.	0	14	19	15
4.	0	16	22	17
5.	0	17	20	15
6.	0	22	16	16
7.	0	17	22	15
8.	0	18	21	15
9.	0	17	23	14
10.	0	18	20	13
11.	0	21	17	15
12.	0	13	19	15
13.	0	17	20	15
14.	0	18	17	16
15.	0	15	18	13
<b>Minimum</b>	0	13	16	13
<b>Maximum</b>	0	22	23	17
<b>Mean</b>	0	17.33	19.53	14.87
<b>SD</b>	0	2.69	2.07	1.19
<b>Median</b>	0	17.00	20.0	15.00
<b>Test for normality (SW Statistic)</b>	–	0.928	0.968	0.883
<b>"p"</b>	–	0.257	0.835	0.053

Extract	Mean	Standard Deviation
T. Chebula ethanolic	17.33	2.69
T. Chebula aqueous	14.87	1.19
Chlorhexidine	19.53	2.07
Distilled water	0	0



**Chlorhexidine > Terminalia chebula ethanolic > T.chebula aqueous > Distilled water**

### Discussion

Antibacterial activities of *T. chebula* extracts against several bacterial strains have been reported. The extract of *T. chebula* showed broad spectrum antibacterial activity<sup>8,9,10</sup>. It has been reviewed as an effective antiviral, antifungal, antibacterial, antioxidant, antiplasmodial, antiamebic, antimutagenic and anticarcinogenic agent<sup>9,11,12</sup>. The fruit has also been evaluated for its efficacy against dental caries pathogens (*Streptococcus* species) in a study<sup>4</sup>. It has been used because a number of phytochemical constituents have been found to be associated with the plant extract that include mainly the different types of chebulic acid, gallic acid, ellagic acid, tannins, amino acids and flavinoids. These compounds found to be responsible for many pharmacological activities<sup>13</sup>. *E. faecalis* associated with post treatment disease is a gram positive cocci that can survive harsh environments. It has been speculated that presence of *E. faecalis* is encouraged by the conventional endodontic techniques mainly calcium hydroxide for root canal dressing<sup>7</sup>. In the present study 2% Chlorhexidine was used as test specimen as an intracanal medicament due to its known effectiveness in eradication of *E. Faecalis*<sup>14</sup>. Due to its established effects chlorhexidine has been used as a test specimen in various comparative evaluations to determine the efficacy of newer materials under research as in few studies<sup>15,16,17</sup>. Barbin et al studied the generations of both parachloroaniline and reactive oxygen species in chlorhexidine which is possibly carcinogenic<sup>18</sup>. The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted research outlook in direction of herbal alternatives. Their beneficial effects allied to the current world wide “back to nature” trend have led to greater attention being paid to these products<sup>19</sup>. The results of the present study are in agreement with a study conducted by Amanullah et al in 2010, they tested the antibacterial activity of *Triphala* and its individual components *T. chebula*, *T. belericia* and

*Emblica officinalis* against various bacterial isolates including *E. faecalis* from HIV infected patients. The study showed that *T. chebula* has highest phytochemical content followed by other components<sup>5</sup>. In the present study ethanolic extract of *T. chebula* is found more effective than aqueous extract (both of them were found less efficacious than 2% chlorhexidine). This may be due to less solubility of the active constituents in aqueous medium, as many of the antibiotic compounds already identified in herbs are aromatic or saturated organic molecules, ethanol is the ideal solvent<sup>20</sup>. Under the various limitations of this in vitro studies following conclusions were made.

### Conclusion

Under the limitations of present study *T. chebula* fruit extracts were found to be effective against *E. faecalis*. Ethanolic extract is more efficacious than an aqueous extract. Further studies are thus indicated to pursue the characterization of active principles, appraise the efficacy of *T. chebula* fruits in terms of concentrations, mode of application to optimize the observed activity and hence its potential use in endodontic.

**Conflict of Interest: None**

**Source of Support: Nil**

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