

Research Article

A Study on the Antibacterial and Cytotoxic Properties of Aqueous Extract of *Terminalia chebula* Fruits

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A B S T R A C T

Introduction: The Combretaceae family species *Terminalia chebula* is frequently referred to as the “king of medicine” utilised throughout Tibet to treat a variety of conditions like diabetes, depression, leprosy, cardiovascular disease, inadequate memory etc due to its alluring antibacterial, antifungal, anticariogenic, anti-inflammatory, antimicrobial activity. *Terminalia chebula* fruits are rich in tannins, flavonoids, and essential oils.

Aim: To study the antibacterial and cytotoxic characteristics of *Terminalia chebula* aqueous extract

Method: Aqueous extracts of *Terminalia chebula* were tested using a brine-shrimp lethal assay for cytotoxicity and antimicrobial activity. In this present study on antibacterial activity, the effectiveness of *T. chebula*'s aqueous fruit extract against gram +ve and gram -ve bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) was assessed using the Agar well diffusion method at different concentrations.

Results: Aqueous *Terminalia chebula* showed good effects as both antibacterial and cytotoxic using brine shrimp lethality assay.

Conclusion: *Terminalia chebula* aqueous extract showed an efficient antibacterial and cytotoxic effect and hence can be used for different biomedical applications.

Keywords: Antibacterial, Cytotoxicity, Aqueous Extract of *T. chebula* (AETC), *S. aureus*, *Pseudomonas*

Introduction

Chebulae fructus, also known as *Terminalia chebula*, is a member of the Combretaceae family, which has its roots

in India. In the mouth, saliva, acid, germs, and food waste combine to generate a sticky substance called “plaque” that sticks to the teeth. This not only leads to tooth decay but

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also results in the difficulty of elimination of plaque.¹ It is generally known that medicinal plants have an antibacterial effect on oral microorganisms.^{2,3} Dried *Terminalia Chebula* fruit is used to cure a variety of ailments, such as dermatitis, diabetes, cardiovascular diseases, urinary tract diseases, and liver disorders.⁴⁻⁶

Studies on the fruit *Terminalia chebula* conducted in vitro and *in vivo* revealed the fruit's therapeutic benefits, such as its antimutagenic properties, antidiabetic, antiproliferative, antioxidant, antimicrobial, and hepatoprotective activities, and the reported toxicity data for this plant are sparse.⁷⁻¹²

Traditional remedies for asthma, sore throat, vomiting, hiccup, diarrhoea, bleeding piles, gout, and heart and bladder problems included the dried, ripe fruit of the *T. chebula* plant.¹³

Numerous gram-positive and gram-negative human pathogenic bacterial species were susceptible to *Terminalia chebula*'s antibacterial properties. Additionally, it has antiviral and antifungal effects.¹⁴

The most prevalent inflammatory diseases brought on by plaque are gingivitis and periodontitis. The most prevalent anaerobic gram-negative bacteria in the subgingival area are *Tannerella* for synthesis, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Prophyromonas gingivalis*, and *Tannerella* for osmoregulation.¹⁵

The *T. chebula* aqueous extract minimised the microbial population and was found to be a powerful anti-cariogenic agent.¹⁶ *T. chebula*'s aqueous extract has the capacity to prevent the growth of cancer cells in culture.¹⁷

In this present study, the antibacterial and cytotoxic efficacy of *Terminalia chebula* aqueous extract has been evaluated.

Materials and Method

Plant Extraction and Sample Preparation

The pulverised dried fruit of *Terminalia chebula* was obtained from Poonamalle, Tiruvallur District, India. The Saveetha Dental College and Hospitals in Chennai provided the *Staphylococcus aureus* and *Pseudomonas aeruginosa* and other human oral pathogens used in this investigation.

Fruit Aqueous Extract Planning

5 g of dried fruit powder of *T. chebula* was weighed and dissolved in 100 mL of distilled water, heated to 50 °C in a heating mantle for 1 hour. The mixture was allowed to cool to room temperature, filtered using Whatman No. 1 filter paper, and then the filtered solution was stored in an airtight container and refrigerated for further experiments.

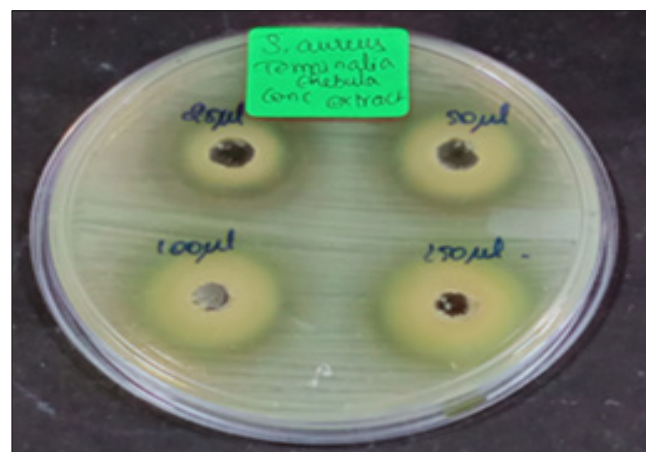
Antimicrobial Activity Evaluation

The antimicrobial capacity was assessed using the agar-based well diffusion technique of *Terminalia chebula* aqueous extract using *Pseudomonas aeruginosa* and

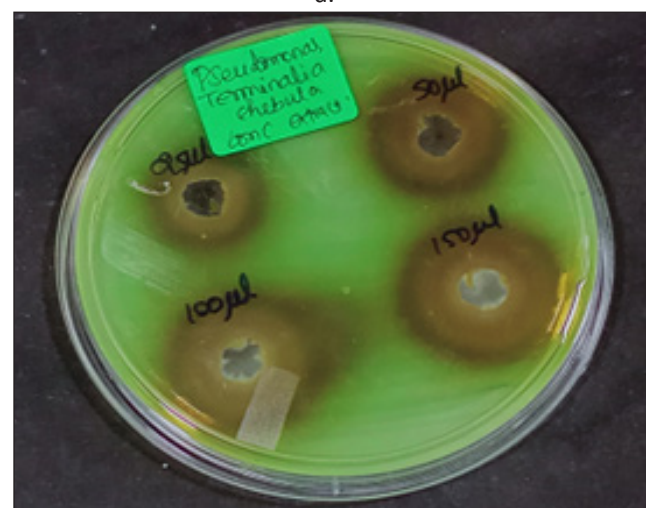
Staphylococcus aureus. Different concentrations of the freshly prepared bacterial suspension were spread evenly over the entire surface of nutrient agar plate extract (25, 50, 100 and 150 µL) was added to the wells, and the resulting plates were then incubated for 24 hours at 37 °C. After 24 hours, the area of inhibition in each plate was recorded.

Evaluation of Cytotoxicity by Brine Shrimp Lethality Assay

5 g of sea salt was measured using an electronic weighing scale and 500 mL of distilled water was combined. Five wells were designated as 5 mL, 10 mL, 20 mL, 40 mL, and 80 mL, respectively, in a medium along with one well marked as control. The wells were filled with prepared salt solution and 10 nauplii were added to each well respectively. Then the prepared *Terminalia chebula* aqueous extract was added to each well with respective measurements using the micropipette. The plate is kept undisturbed and observed after 24 hours.



a.



b.

Figure 1. Zone of inhibition for the aqueous extract of *Terminalia chebula* (a) *Staphylococcus aureus* (b) *Pseudomonas aeruginosa*

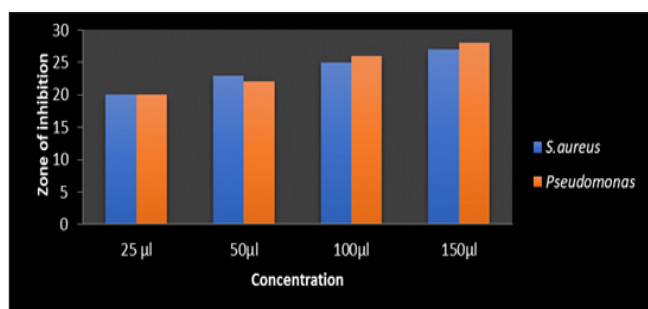


Figure 2. Comparison of Antibacterial activity of Terminalia chebula Fruit Extract against Oral Pathogens

Results and Discussion

Antibacterial Action

The Agar well diffusion method was used to conduct the antibacterial activity test. For determining the inhibitory action over *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two agar plates were employed, respectively. Each plate included four wells with varying plant extract concentrations, such as 25 µL, 50 µL, 100 µL, and 150 µL. The diameter of the detrimental zone against *Staphylococcus aureus* measured 20 mm, 23 mm, 25 mm, and 27 mm, respectively. The diameter of the plant extract's detrimental zone against *Pseudomonas aeruginosa* was 20 mm, 22 mm, 26 mm, and 28 mm. When compared to other concentrations, 150 µL concentration produced the highest activity for both organisms. Figure 1 depicts the antibacterial activity of Terminalia chebula's aqueous fruit extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. When used against oral infections, the aqueous extract demonstrated effective antibacterial properties as shown in Figure 2 and Table 1.

Table 1. Results Indicating the Fruit Extract from Terminalia chebula, which Contains S. aureus and P. aeruginosa, having Antibacterial Activity

Concentration (µL)	<i>S. aureus</i>	<i>P. aeruginosa</i>
25	20	21
50	24	23
100	25.2	26.8
150	27.8	29.2

Cytotoxicity by Brine Shrimp Lethality Assay

In order to test for cytotoxicity, brine prawn nauplii were used. Each well contained ten nauplii with aqueous extract concentrations of 5, 10, 20, 40, and 80 mL. The LD50 concentration was attained at 80 L concentration, and half of the nauplii in each well survived during incubation of 24 hours. Results are shown in Figures 3 and 4 and Table 2.

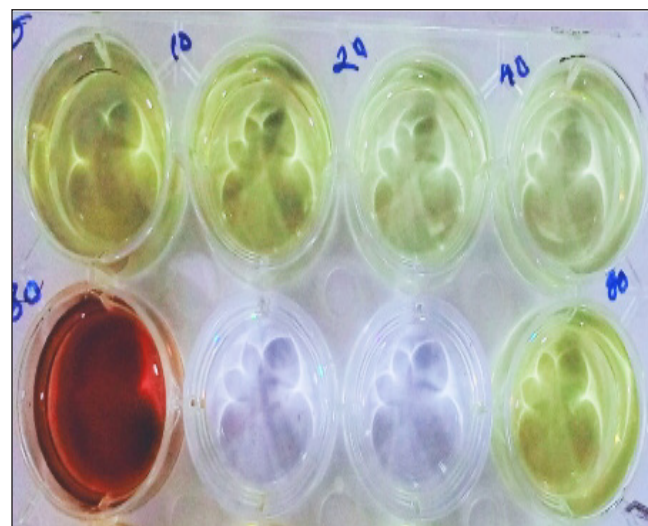


Figure 3. Cytotoxic effect of Terminalia chebula fruit extract with varying concentrations

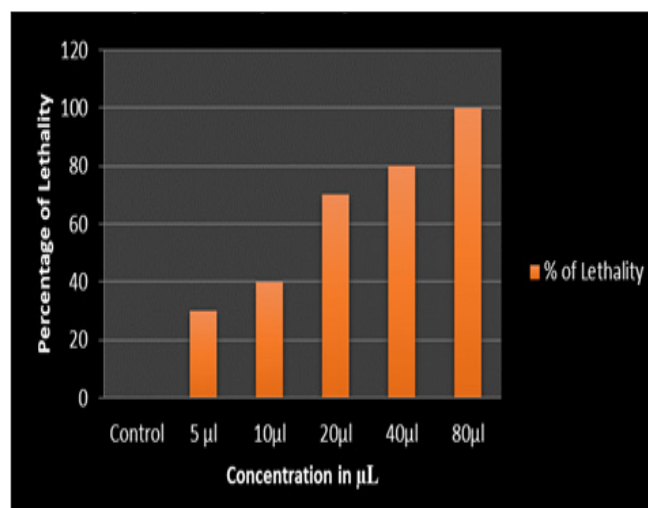


Figure 4. Percentage of Lethality against Different Concentrations of Aqueous Extract of Terminalia chebula Fruit

Existing research works stay concordant with the current cytotoxic activity results.¹⁸ The fruits of *Terminalia chebula* have a high content of tannings which is hydrolysable Pyrogallol type which has enormous activity and other constituents such as polyphenols.¹⁹ Besides antimicrobial activity, *T. chebula* also has antimutagenic activity. It is a putty-like plant used for various pharmacological effects.²⁰ The methanolic and aqueous extract of leaves also produced enormous antibacterial activity against gram-negative bacteria.²¹ The traditional preparations of *T. chebula* have various properties like anti-ageing activity and topical applications. If the dose of *T. chebula* increases, it causes digestive discomfort.²² *Terminalia chebula* fruit aqueous extract can be used as a potential anti-cariogenic mouthwash and in gel or varnish form.²³⁻²⁵

Table 2. Outcome of the Aqueous Extract of *Terminalia chebula* Fruits' Lethality for Brine Shrimp

Dose (ppm)	Initial Nauphili	Number Survived after 24 Hrs	Number Died after 24 Hrs	Average Numbers Died after 24 Hrs	% Mortality/ Lethality
5	10	9	1	1	10
10	10	8	2	2	20
20	10	5	5	5	50
40	10	2	8	8	80
80	10	0	10	10	100

Conclusion

The aqueous extract of *Terminalia chebula* fruit used in this investigation displays extremely high antibacterial action against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The direct anti-viral activity of the 50% ethanolic extract prepared from the fruits of *Terminalia chebula* on Herpes Simplex Virus-2 (HSV-2) viruses has also been reported. With regard to *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the aqueous fruit extract of *Terminalia chebula* has demonstrated good antibacterial activity in the current study. Because these medications are plant-based, they can be regarded as being safe for humans and less hazardous, and they can be employed for further research.

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Conflict of Interest: None

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