



Screening of Isolated Tannins from *Terminalia chebulla* against Human Pathogens

Shinde Saheb L

¹Department of Botany and Horticulture
Yeshwant Mahavidyalaya, Nanded (M.S.) India.
E-mail: sahebshinde4@gmail.com

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Abstract

The tannins isolated from *Terminalia chebulla* were screened against *Escherchia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Shigella boydii*, *Shigella flexneri*, *Staphylococcus aureus* and *Staphylococcus epidermidis* using agar well diffusion method. The tannins showed antibacterial properties against all the test bacteria. The tannins extracted from bark were more antibacterial than the tannins from leaves. Out of all tested bacteria *Bacillus subtilis* was more inhibited. The minimum inhibitory concentration (MIC) of the tannins ranged between 5 to 8 mg/ml.

INTRODUCTION

Many plant extracts and essential oils isolated from plants have been shown to possess biological activity *in vitro* and *in vivo*, which justifies research on plant based medicine focused on the characterization of antimicrobial activity of these plants. Thus the use of medicinal plants in disease treatment and prevention can also be seen as prehistoric and their present use can be supported by the traditional optimization of their application in disease control. Medicinal uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts from the plants. These plant extracts are a source of many potent and powerful drugs. Brazil, Cuba and India are examples of countries that have a diverse flora and rich tradition in the use of medicinal plants both as antibacterial and antifungal applications

Tannin is general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution. Tannins can be toxic to bacteria, fungi and yeast (Harborne, 1973). Tannins have been isolated

from some plants and they exhibited antibacterial properties (Mitcher *et al.*, 1988; Vasait and Khandare, 2017).

E. officinalis and *Coriandrion sativum* are inhibitory to gram negative bacteria (Sahabat and Prween, 2007). The *Embilica officinalis*, *Terminalia chebula*, and *T.chebulla* have antimicrobial properties and inhibited the growth of *Streptococcus mutans* and gram positive cocci (Jagdish *et al.*, 2009). The species of combretaceae and pomegranate showed antibacterial properties because of the presence of tannins (Shinde *et al.*, 2009; Alne and Swami, (2016).

Terminalia chebulla with antibacterial property is being used extensively in Indian system of medicine (Sabu and Kuttan, 2002; Mosoko *et al.*, 2005; Patni *et al.*, 2005). *Embilica officinalis* screened for its potential antibacterial activity against pathogenic bacterial strains. The fruit of *E. officinalis* is commonly known as amla and is highly valued in traditional Indian medicine (Scartezzini *et al.*, 2006).

The fruit of *E. officinalis* is antimicrobial (Anonymous, 2006). Several constituents of *E. officinalis* bark have been identified, mainly the hydrolysable tannins, emblicanin A, emblicanin B, pignuconin and pedunculagin (Perianayagam *et al.*, 2005; Chaudhari *et al.*, 2006). Emblicanin A and B have been proposed to be the active constituents with significant *in vitro* antimicrobial properties (Ghosal *et al.*, 1996).

MATERIAL AND METHODS

Collection of plant materials

The leaves and bark of test plant *Terminalia chebulla* were collected from Nanded region of Maharashtra state.

Source of microorganisms

The bacteria selected for study were common human pathogens like *E. coli* (ATCC-10412), *Pseudomonas aeruginosa* (ATCC-27853), *Bacillus subtilis*, *Shigella boydii*, *Shigella flexneri*, *Staphylococcus aureus* (ATCC-103207) and *Staphylococcus epidermidis*. They were obtained from Microbiology Department of Yeshwant Mahavidyala, Nanded, (M.S.).

Standardization of microorganisms

For standardization of microorganism exactly 0.5 ml of overnight cultures of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 3 hours to standardize the culture to 10^6 cfu/ml. A loop-ful of the standard culture was used for the antibacterial activity (Collins *et al.*, 1995)

Extraction of tannins

The powdered leaves and fruit samples (5g) were boiled in 10 ml of distilled water for 3 minutes on a hot plate. The mixture was filtered while hot and the resulting filtrate was used to carry out ferric chloride test (Trease and Evans, 1983). Sample of the filtrate (1 gm) was weighed in to a beaker and 10 ml of distilled water added. This was boiled for 5 minutes. Two drops of 5% ferric chloride (FeCl_2) was than added. Production of greenish precipitate indicated the presence of tannins (Trease and Evans, 1983).

Assessment of antibacterial activity of plant extracts

Antibacterial activity test was evaluated by Agar well-diffusion method and expressed by diameter zone of inhibition in mm. The bioassay was carried out by using 1 ml of inoculums prepared from an overnight culture for given test bacterium, 1 ml of the bacterial cell suspension was poured in the Petri plate and the plates were poured

with respective medium. The medium was allowed to solidify and wells were prepared using sterilized cork borer (diameter 5 mm). The cuts agar disks were carefully removed by the use of forceps sterilized by flaming. Each well was filled with different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 etc. mg/ml) of tannins isolated from the plant extract. Plates were then incubated aerobically at $28 \pm 2^\circ\text{C}$ for 24 hrs. The experiments were conducted with three replications. The zones of inhibitions were then recorded.

Determination of minimum inhibitory concentration

Isolated tannins from *T.chebulla* were used to check MIC. Different concentrations of tannins ranging between 5 to 8 mg/ml were introduced with an overnight culture of *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Shigella boydii*, *Shigella flexneri*, *Staphylococcus aureus* and *Staphylococcus epidermidis* diluted to give a final concentration of 10^6 cfc/ml. The tubes were incubated at $28 \pm 2^\circ\text{C}$ for 24 hrs. The least concentration of tannin that did not permit any visible growth of the inoculated test bacteria in broth culture was regarded as minimum inhibitory concentration (MIC) in each case (Collins *et al.*, 1995).

RESULTS AND DISCUSSION

The isolated tannins from leaves and bark of *T.chebulla* in this study showed antibacterial activity against *E.coli*, *P. aeruginosa*, *B. subtilis*, *S. boydii*, *S. flexneri*, *S. aureus* and *S. epidermidis* (Table 1 and 2). In table 1, it was revealed that different concentrations of *Terminallia bellerica* leaves extracts caused significant effects against all test bacterial pathogens. As the concentration increases inhibitory activity was increased. Table 2, showed that different concentration of bark extract also caused significant inhibitory effects against all test bacterial pathogen.

The tannins isolated from bark exhibited maximum antibacterial activity than leaves tannins. The antibacterial activity increases with increase in concentration of tannins isolated in this study. According to Kurosaki and Nishi, (1983) the concentration increases the antibacterial activity also increases.

Out of all bacteria tested in this study *B. subtilis* showed maximum inhibition in both tannins isolated from leaves and bark while *S. epidermidis* showed least inhibition.

Table 1. Antibacterial effects of tannin isolated from leaves of *Terminalia bellerica*

Conc. (mg/ml)	Ec	Pa	Bs	Sb	Sf	Sa	Se
0.5	0	0	0	0	0	0	0
1.0	2.2	2.2	3.3	4.4	2.3	3.2	3.6
1.5	7.4	8.3	7.4	8.3	5.4	6.3	6.0
2.0	9.3	9.0	8.3	8.7	7.9	6.9	8.0
2.5	9.7	9.3	9.3	9.3	9.0	8.4	8.3
3.0	10.3	10.3	10.4	10.9	9.3	8.8	8.5
3.5	12.9	10.8	10.9	10.4	10.5	9.0	9.5
4.0	13.4	14.8	12.8	12.5	10.5	9.3	10.9
4.5	14.9	14.5	16.8	13.3	11.4	10.7	12.4
5.0	14.0	14.1	17.3	13.4	11.5	11.9	13.4

Ec-*E. coli*, Pa-*Pseudomonas aeruginosa*, Bs-*Bacillus subtilis*, Sb-*Shigella boydii*, Sf-*Shigella flexneri*, Sa-*Staphylococcus aureus*, Se-*Staphylococcus epidermidis*.

Table 2. Antibacterial effects of tannin isolated from bark of *Terminalia bellerica*

Conc. (mg/ml)	Ec	Pa	Bs	Sb	Sf	Sa	Se
0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.5	2.3	1.3	3.0	1.4	0.0	0.0	1.6
2.0	2.4	3.3	4.8	2.8	1.6	2.0	1.8
2.5	4.7	3.0	5.2	4.0	4.8	2.8	2.8
3.0	5.6	3.0	7.4	5.8	5.7	2.8	4.8
3.5	5.9	4.3	7.3	6.2	6.1	5.0	5.2
4.0	7.2	5.1	9.5	8.3	6.9	5.6	6.2
4.5	8.0	5.9	10.3	8.3	7.0	6.0	6.2
5.0	11.4	7.9	12.8	8.3	8.1	6.9	6.6

Ec-*E. coli*, Pa-*Pseudomonas aeruginosa*, Bs-*Bacillus subtilis*, Sb-*Shigella boydii*, Sf-*Shigella flexneri*, Sa-*Staphylococcus aureus*, Se-*Staphylococcus epidermidis*.

Table 3. Minimum inhibitory concentration (MIC) of tannins isolated from leaves and bark of *Terminalia bellerica*

Organism	MIC (mg/ml) of leaf tannins	MIC (mg/ml) of fruit tannins
Ec	6.0	6.5
Pa	6.5	7.0
Bs	4.5	5.0
Sb	7.0	7.5
Sf	6.5	8.0
Sa	7.0	8.0
Se	7.8	7.5

Ec-*E. coli*, Pa-*Pseudomonas aeruginosa*, Bs-*Bacillus subtilis*, Sb-*Shigella boydii*, Sf-*Shigella flexneri*, Sa-*Staphylococcus aureus*, Se-*Staphylococcus epidermidis*.

The *T.chebulla* plant which is rich in tannins has been shown to possess antimicrobial properties against test microorganisms this study (Banzouzi *et al.*, 2004; Karou *et al.*, 2003; Banso and Adeyemo, 2007) observed the antimicrobial activity is due to tannins, alkaloids and glycosides.

According to Khandare and Vasait, (2017) the antimicrobial activity of plant extracts of some medicinal plants at different concentrations was assessed and got better inhibitory effects as the concentrations of extract was increased.

Alne and Swami, (2016) used plant extracts of *Punica granatum* L. against bacteria. According to Vasait and Khandare, (2017) the antimicrobial activity of medicinal plant carried out by using different solvents. All the solvent extracts shown antibacterial activity.

The minimum inhibitory concentration (MIC) of tannins isolated from leaves and bark in this study against the test bacteria ranged between 5 to 8 mg/ml (Table 3). Antimicrobial agents with low activity against an organism showed a high MIC while a high active antimicrobial agent showed slow MIC.

CONCLUSION

The result of the present study suggests that the tannins Isolated from leaves and bark of *T.chebulla* possessed remarkable antibacterial activity. Thus there is possibility of developing the plant as a source of antimicrobial agent.

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