



Antibacterial activity of *Terminalia chebula* Retz., against *Escherichia coli*

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Abstract

Terminalia chebula Retz. belongs to the family Combretaceae and is commonly known as Black myrobalan. In the present study fruits of *Terminalia chebula* were screened for potential antibacterial activity against Gram-negative bacterial strain *Escherichia coli*. The antibacterial activity was determined in aqueous, methanol, ethanol and acetone extracts by using disc diffusion (Kirby-Bauer) method. The activity was carried out for different concentrations of plant extracts i.e. 100, 200 and 400 mg/ml and the relative solvents were used as control for respective extracts. Ciprofloxacin was used as positive control. The antibacterial activity of *Terminalia chebula* gave encouraging results.

Keywords Antibacterial, *Terminalia chebula*, *Escherichia coli*, Black myrobalan

Introduction

Escherichia coli is one of the bacterial species found in normal microflora of the digestive tract. However, after 1940, the *E.coli* strains have been reported to cause several diseases, one of which was *E.coli* enteropathogenic (EPEC) (Nataro and Kaper, 1998). EPEC is the most frequent cause of diarrhoea in babies and infants all over the world in general and in developing countries like Indonesia in particular and South East Asia and cause infection by way of adhesion with the receptor present on the host cell surface, like in the ileum part of intestine epithelial cell. Adhesion is a factor of bacteria virulence carried out by the adhesion protein present in pilli and outer membrane protein (OMP) of bacteria. Bacterial adhesion on the tissue can determine the microorganism colonization capability (Suroño, 2004). According to Todar (2008) bacterial adhesion which is followed by the occurrence of colonization in the sensitive host is an important factor and is needed to start the pathogenesis of diseases.

The Herbals have become increasingly popular and their use is wide spread clear-cut proof of their efficacy on microorganisms inducing pathogens is yet to be explored. Various medicinal plants have been used for years in daily life to treat diseases all over the world.

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Higher plants as source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoural and antimicrobial agents (Farombi, 2003). *Terminalia chebula* plant has been selected to determine its antibacterial property. The study looks into the *in vitro* antibacterial activity of this plant with different solvent extracts against Gram-negative pathogenic bacterium *E.coli* that causes the most common cases of infectious diseases.

Material and Methods

Collection and Identification of plant material

The dried fruits of *Terminalia chebula* were collected from VHCA, Karnal and identified by using standard floras like 'The Flora of British India' (J.D. Hooker) and compared with standard voucher specimens of herbarium.

Preparation of Plant extracts

Aqueous extract

50g of powdered plant material was mixed well in 500 ml distilled water with continuous stirring for



30 minutes. The solution was kept at room temperature for at least 24 hours and then filtered using muslin cloth. The supernatant was again filtered using Whatman filter paper No.1 under strict aseptic conditions. Then the extract was evaporated to dryness at its boiling temperature of 100 °C. The extract was collected in fresh sterilized glass vials and stored at 4 °C until use (Akueshi *et al.*, 2002). Aqueous extract was prepared in concentration of 100, 200 and 400 mg/ml by dissolving the extract in distilled water.

Organic Solvent Extraction

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50g of powdered material was extracted in Soxhlet extraction apparatus with 500 ml of each of the following solvents; methanol, ethanol and acetone. The extracts obtained with each solvent were filtered through Whatman filter paper No. 42 and the respective solvents were evaporated (at 40 °C) with the help of heating mantle. The sticky dark coloured substances were obtained and stored in refrigerator for prior to use following Beyer and Walter (1997); Dhale and Markandeya (2011) and Acharyya *et al.*, (2009). For antibacterial analysis different concentrations of extracts viz., 100, 200, 400 mg/ml were prepared by dissolving the residues in the respective solvents. Extracts were stored in glass vials at 4°C for further use.

Collection of Bacterial culture

The test organism *Escherichia coli* (MTCC 723) culture was procured from IMTech, Chandigarh and maintained on nutrient agar slants.

Antibacterial Susceptibility Assay

In vitro antibacterial activity of afore said crude extracts of selected medicinal plants was evaluated using the disc diffusion method of Bauer *et al.*, (1966). 100µl of each bacterial culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Muller Hinton agar plated evenly using a sterile spreader. The plates were dried for 15 minutes and then used for the sensitivity test. The discs with different concentrations (100, 200 and 400 mg/ml) of plant extracts were placed on the Muller-Hinton agar surface. The solvent loaded disc without extracts in it served as negative control in the study. The four discs were placed in each test plate, one of which was negative control and three were treated ones. The negative control was pure solvent of respective plant extract. Besides, the

controls, each plate had three treated discs placed at equidistance to each other. The plate was then incubated at 37°C for 18 to 24 hours. After the incubation, the plates were examined for inhibition zone. The inhibition zone was then measured in millimeters using transparent scale of Hi-media. All tests were performed in sterile condition and repeated three times to ensure reliability (Erturk *et al.*, 2003; Ncube *et al.*, 2008; Segni *et al.*, 2011).

Minimum inhibitory Concentration

Determination by broth micro dilution method

Minimum inhibitory concentration (MIC) of active crude extracts was determined by broth microdilution method as described in NCCLS (1997). The test was performed in 96 wells microtiter plates, two- fold serial dilutions of all extracts and standard antibiotics were made in Cation Adjusted Muller-Hinton Broth (CAMHB) ranging from 1-512 µg/ml. Inoculum was prepared in the same medium at a density adjusted to 0.5 McFarland standard and added 100µl of it to each well except negative control. The final volume in the well was 200 µl. After 24 hours of incubation at 37°C the MIC was calculated as the visible lowest concentration of the extract inhibiting growth of bacterial strain (Kaushik and Chauhan, 2009).

Minimum Bactericidal Concentration determination

MBC was determined by sub culturing the 5 µl of test dilution from each well on to a nutrient agar plate and incubating further at 37°C for 24 hours. The complete absence of growth at applied concentration was considered as the minimum bactericidal concentration (Seric *et al.*, 2009).

Statistical Analysis- Statistical analysis of data was carried out by using analysis of variance (one way ANOVA) followed by Zar (1999).

Results and Discussion

The four different plant extracts of *Terminalia chebula* with their three different concentrations i.e. 100, 200 and 400mg/ml were subjected to check the antibacterial activity of all the four fruit extracts against *Escherichia coli*. Aqueous extract gave no activity at the concentration of 100mg/ml but the methanol, ethanol and acetone extracts showed 4.0mm, 7.0mm and 4.0mm zone of inhibition respectively, at the concentration of 100mg/ml. At



the concentration of 200mg/ml, all four (Aqueous, methanol, ethanol and acetone) extracts showed 2.3mm, 6.6 mm, 10.0mm and 6.0mm zone of inhibition respectively. At the concentration of

400mg/ml all above mentioned extracts showed 3.3mm, 10.0mm, 14.3mm and 8.0 mm zone of inhibition respectively and the difference was highly significant.

Thus ethanol fruit extract of *Terminalia chebula*

Retz. showed best activity i.e. 7.0 mm, 10.0 mm and 14.3 mm zone of inhibition at the concentration of 100, 200 and 400mg/ml respectively against *E.coli* (Table 1). Negative control showed no activity and Positive control (Ciprofloxacin) gave zone of inhibition at the concentration of 5µg/ml was 22.0 mm. The MIC value of *Terminalia chebula* ranged from ≤ 1 to 512µg/ml and the MBC value ranged from 2 to 512µg/ml (Table 2).

Table 1. Antibacterial activity of different plant extracts of *Terminalia chebula*

Plant extracts	Effective Zone of inhibition at different concentration(mm)		
	<i>E.coli</i>		
	100 mg/ml	200 mg/ml	400 mg/ml
Aqueous extract	0.0±0.0	2.3±0.57	3.3±0.57
Methanol extract	4.0±1.0	6.6±0.57	10.0±0.0
Ethanol extract	7.0±1.0	10.0±0.0	14.3±0.57
Acetone extract	4.0±0.0	6.0±0.0	8.0±0.0
	(F=99, df=3,8 ,P>0.001)	(F=177.8, df=3,8 ,P>0.001)	(F=375.16, df=3,8 ,P>0.001)

Effective zone of inhibition = Zone of inhibition – Disc diameter, ± = Standard deviation

Table 2. The MIC and MBC of *Terminalia chebula* against *E coli*

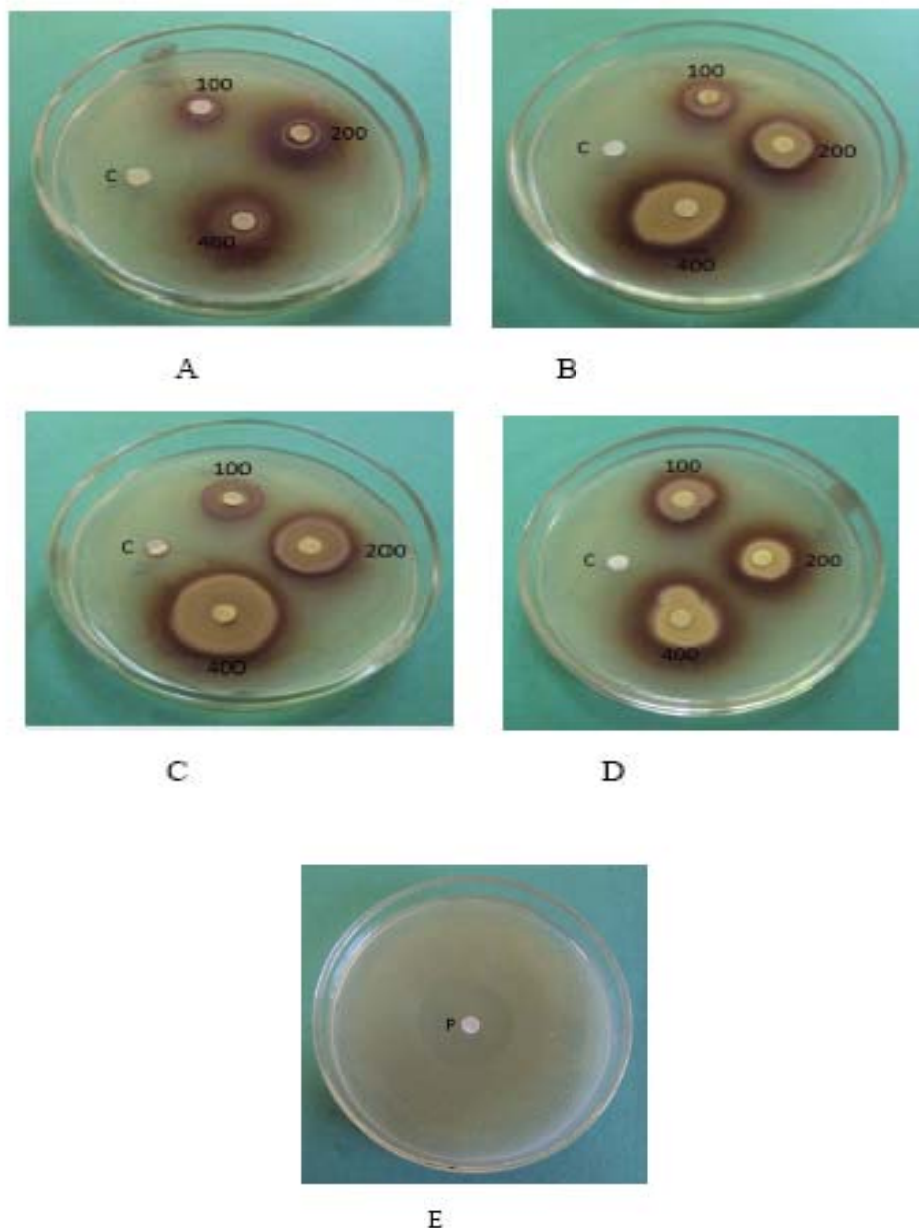
Plant extracts	MIC(µg/ml)	MBC(µg/ml)
Aqueous extract	ND	ND
Methanol extract	256	256
Ethanol extract	64	64
Acetone extract	512	512
Ciprofloxacin	≤1	2

ND= Not Done, MIC=Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration

According to Johnson (1991) *E. coli*, though normally a gut commensal, has attracted the clinical significance owing to the recognition of several strains of diarrhoeagenic *E.coli* which distinct virulent factors and also an important organism in urinary tract infections (UTIs). The aqueous and ethanol extracts of *Azadirachta indica* has strong

(IZD=18mm and 20mm) inhibitory action against *S. aureus* but failed to show any promising (IZD=8mm or more) inhibitory effect against the Gram-negative bacteria *P.aeruginosa* and *E.coli*. The aqueous extract of *T. chebula* and *A. marmelos* showed strong (IZD=15-23mm) inhibitory action against all the strains evaluated. The MIC value of *T. chebula* and *A. marmelos* against the test strains ranged from 1.56 to 3.12mg/ml and 3.12 to 6.25mg/ml, respectively. The MBC values for *T. chebula* ranged from 1.56 to 6.25mg/ml and for *A. marmelos* showed strong (IZD=15-23mm) inhibitory action against all the strains evaluated. The MIC value of *T. chebula* and *A. marmelos* against the test strains ranged from 1.56 to 3.12mg/ml and 3.12 to 6.25mg/ml, respectively. The MBC values for *T. chebula* ranged from 1.56 to 6.25mg/ml and for *A. marmelos* from 12.50 to 50.0mg/ml (Chattopadhyay *et al.*, 2009). Suriya *et al.* (2012) worked on antibacterial activity of *Abutilon indicum*, *Hygrophila spinosa* and *Mimosa pudica* were studied by agar well diffusion method in vitro.





Zone of inhibition exhibited by different extracts of *Terminalia chebula* against *E. coli*

A. Aqueous extract **B.** Methanol extract **C.** Ethanol extract **D.** Acetone extract **E.** Ciprofloxacin

Petriplates: 100, 100 mg/ml; 200, 200 mg/ml; 400, 400mg/ml; C, Negative Control; P, Positive control.

The effect of antibacterial potential was examined against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Vibrio cholera*, *Salmonella typhi* and *Salmonella paratyphi*. The

methanol extract of these medicinal plants have showed consistently significant inhibitory activity on different bacterial pathogens tested. Furthermore, the Minimum Inhibitory Concentration carried out by broth dilution assay, ranged between 0.2 to 0.9 mg/ml. Overall the

methanol extract was found more effective. The results of the extracts were compared with the standard antibiotic Kanamycin.

Conclusion

In the current investigation, the methanol, ethanol and acetone extracts of *Terminalia chebula* were found active but the highest antibacterial activity was observed in ethanol extract against test organism *E.coli*. The present study justified the claimed usage of fruits in the traditional system of medicine. However, more work is needed for better evaluation of the potential effectiveness of crude extracts as the antibacterial agents.

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