Evaluation of Antimicrobial Activity and Phytochemical Analysis of Antidiabetic Plant *Terminalia chebula*

Gaurav Kumar, Ram Kumar Pundir* and Satish Rana

Department of Biotechnology Engineering, Ambala College of Engineering and Applied Research (ACE), Devsthali, Near Mithapur, P. O. Sambhalkha-133101, Ambala, Haryana, India

*Author to whom correspondence should be addressed; E-Mail: drramkpundir@gmail.com; Tel.: 0171-2821833; Fax: 0171-2822002.

Article history: Received 19 November 2012, Received in revised form 6 January 2013, Accepted 8 January 2013, Published 10 January 2013.

Abstract: The present investigation was undertaken to evaluate in vitro antimicrobial activity and phytochemical analysis of *Terminalia chebula*. The five extracts (acetone, ethanol, methanol, aqueous cold and hot) of *Terminalia chebula* fruit were screened for their antimicrobial activity against five pathogenic microorganisms (*Bacillus amyloliquefaciens*, *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella enterica* ser. Typhi and *Aspergillus fumigatus*) by using agar well diffusion method. This is the first report on antimicrobial activity of *Terminalia chebula* fruit extract against *Bacillus amyloliquefaciens*, *Staphylococcus epidermidis*, *Salmonella enterica* ser. Typhi, and *Aspergillus fumigatus*. The methanolic extract was found to be most effective against all the tested bacterial and fungal strains with zone of inhibition ranging from 25 mm to 30 mm followed by acetone extract, aqueous(cold and hot)extract, and ethanol extract. The minimum inhibitory concentration was recorded 0.25 mg/mL against the two tested microorganisms (*Bacillus amyloliquefaciens*, and *Aspergillus fumigatus*) and < 0.12 mg/mL against the three tested microorganisms (*Escherichia coli*, *Salmonella enterica* ser. Typhi and *Staphylococcus epidermidis*) using macro-dilution agar plate technique. The phytochemical analysis revealed the presence of flavanoid, tannin, steroid and absence of alkaloid, cardiac glycoside, steroid and polyurinoides. From the present findings, methanolic extract of *Terminalia chebula* may be used to control infectious diseases in diabetes patients.

Keywords: antimicrobial activity; agar well diffusion; phytochemical analysis; minimum inhibitory concentration; *Terminalia chebula*. 
1. Introduction

*Terminalia chebula* is a medium to large sized tree belonging to the family Combretaceae, commonly known as black myrobalan and haritaki. Its other commonly used Sanskrit name, Abhaya, refers to the ‘fearlessness’. It provides in the face of the disease. It is native to Indian subcontinent and the adjacent areas such as Pakistan, Nepal and the South-West of China stretching as far south as Kerala or even Srilanka. *Terminalia chebula* is a flowering evergreen tree attaining a height up to 30 m, with widely spreading branches and a brown roundish crown. The leaves are elliptical, oblong, with an acute tip, cordate at the base, margins entire, glabrous above with a yellowish pubescence below. The flowers are monoecious, dull white to yellow, with a strong unpleasant odour, borne in terminal spikes or short panicles. The fruits are glabrous, ellipsoid to ovoid drupes, and yellow to orange brown in colour. As medicinal value, *Terminalia chebula* is reported to be antimicrobial, hepatoprotective, anti-inflammatory, antidiabetic, immunomodulatory, antioxidantive and adaptogenic.

Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, hyperlipedemia, hyperaminoacidemia, and hypoinsulinaemia. It leads to decrease in both insulin secretion and insulin action (Altan et al., 2003). Diabetes mellitus is a common disorder affecting more than 300 million people worldwide. Before the introduction of modern medicine, disease treatment was entirely managed by medicinal plant. Diabetes mellitus with plant derived compound which can accessible are highly attractive due to their reliable result of antidiabetic activity and play a significant role in improving the hypoglycemic action (Rao et al., 2010).

Above literature revealed that there is a need of the hour to search the new secondary antimicrobial metabolite from the plants. The present study was to evaluate the antimicrobial activity and phytochemical analysis of *Terminalia chebula* fruit extracts.

2. Materials and Methods

2.1. Collection of Plants

In the present study, the fruits of *Terminalia chebula* were collected from Herbal Garden of Ambala Cantt, Haryana, India and their extracts were evaluated for their antimicrobial activity against five pathogenic microorganisms and phytochemical analysis.

2.2. Preparations of Fruit Extracts

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30 °C) for two days and pulverized to a fine powder using a sterilized mixer grinder and stored in airtight bottles. Four different solvents namely ethanol, methanol,
acetone and aqueous (hot and cold) were used for extraction. The 10 g of pulverized fruit was separately soaked in 100 mL of acetone, ethanol, methanol, and cold sterile distilled water for 24 h. In addition, the same amount (i.e. 10 g) of pulverized fruit was immersed in 100 mL of hot sterile distilled water (100 °C) and allowed to stand for 30 min on a water bath with occasional shaking, and then kept undisturbed for 24 h. Each preparation was filtered through a sterilized Whatman No. 1 filter paper and the filtered extract was evaporated in water bath at 60 °C till to finally get dry powder (Bag et al., 2009; Ogundiya et al., 2006).

2.3. Tested Microorganisms

The test microorganisms were Bacillus amyloliqfaciens-MTCC-1488, Staphyl-ococcus epidermidis-MTCC435 (Gram positive), Salmonella enterica ser. Typhi-MTCC3216, Escherichia coli-MTCC723 (Gram negative) and Aspergillus fumigatus-MTCC3216 (Mould).

2.4. Screening for Antimicrobial Activity by Agar Well Diffusion Method

Plate count agar (PCA) plates were inoculated with 100 μL of standardized inoculum (1.5 × 10^8 CFU/mL) of each selected bacterium (in triplicates) and spread with sterile swabs. Wells of 8 mm size were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. The 100 μL - 200 μL volume of different plant extracts (acetone, ethanol, methanol, aqueous cold and hot) were poured into a different wells of inoculated plates. Acetone, aqueous (hot and cold), ethanol, and methanol were used as a negative control which was introduced into a well instead of plant extract. Commercially available antibiotics-tetracycline (antibacterial) and ketoconazole (antifungal) were used as positive control. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar (Rios et al., 1988). After incubation for 24 hrs at 37 °C, the plates were observed. If antimicrobial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters. Antimicrobial activity was recorded if the zone of inhibition was greater than 8 mm (Hammer et al., 1999).

2.5. Determination of Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24 h (Thongson et al., 2004). The MIC of the most promising extract of Terminalia chebula fruit was determined by following the macro dilution agar method.

In the macro dilution agar method, a two-fold serial dilution of most promising extract was prepared in sterile distilled water to achieve a decreasing concentration ranging from 1 to 0.625
mg/mL into five sterile tubes labeled 1 to 5. Sterile cork borer of 8.0 mm diameter was used to bore well in the nutrient agar plates for bacteria and Czapek Dox agar plate for fungus and 100 μL volume of each dilution was added aseptically into the wells made in nutrient agar plates in triplicate that had pathogenic strain seeded with the standardized inoculum (1.5 × 10⁸ CFU/mL). The 100 μL of different solvents introduced into the different well instead of plant extract were used as negative control. All the bacteria containing plates were incubated at 37 °C for 24 hours and fungus plate at 25-27 °C for 3-5 days. Then the plates were observed for growth. The lowest concentration of an extract showing a clear zone of inhibition was considered as the MIC (Pundir et al., 2010).

2.6. Phytochemical Analysis

The crude methanolic extract of *Terminalia chebula* fruit was subjected to qualitative phytochemical screening for the identification of various classes of active chemical constituent using the methods described by Trease and Evans (1987), Karumi et al. (2004), Fasoyira and Adegoke (2007) and Parekh and Chanda (2007).

*Test for alkaloid:* Hager’s test: Methanolic extract was treated with Hager’s reagent (5% picric acid). Formation of yellow colored precipitate indicates the presence of alkaloids.

*Test for saponin:* 5 mL of methanolic extract was shaken vigorously with 10 mL of distilled water for 2 min. The appearance of foam that persisted for at least 15 min or the foaming of an emulsion when olive oil was added that confirmed the presence of saponin.

*Test for steroid:* 2 mL of chloroform was added to 2 mL of methanolic extract by addition of 2 mL of concentrated H₂SO₄ and shaken well. Chloroform layers appearing red and acid layer showing greenish yellow fluorescence colour indicated the presence of steroid in the test extract.

*Test for tannins:* few drops of 5% FeCl₃ solution were added to 2-3 mL of methanolic extract. Appearance of deep black blue colour indicated the presence of tannin.

*Test for flavanoid:* few drops of lead acetate solution were added to 2-3 mL of methanolic extract. Formation of yellow colour indicates the presence of flavanoid.

*Test for cardiac-glycosides:* Keller-killani test: 1 mL of glacial acetic acid was added to 2 mL of methanolic extract followed by addition of few drops of FeCl₃ solution and concentrated H₂SO₄. Development of green blue colour indicates the presence of cardiac glycosides.

*Test for poly-urinoides:* crude methanolic extract was added drop wise into a test tube containing 10 mL of C₂H₅OH and the tubes were observed for the appearance of violet or blue precipitate.
3. Results and Discussion

Medicinal plants have been considered a boon to human society to cure a number of ailments. Plants have a great potential of producing natural drugs that have been the source of most of the active ingredients of medicines, as they are non-toxic, having no side effects and easily available (Bandow et al., 2003). Several works have been documented on the pharmacological screening of plant extracts which have been exploited as the source of innumerable therapeutic agents (Herrera et al., 1996; Natranjan et al., 2003; Sakagami et al., 2001).

The antibacterial activity of different solvent extracts of *Terminalia chebula* fruit was evaluated against *Bacillus amyloliquefaciens*, *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella enterica* ser. Typhi and *Aspergillus fumigatus*, and the results are shown in the Table 1 and Fig. 1.

![Figure 1](image1.png)

**Figure 1.** Plates showing antimicrobial activity of fruit extracts of *Terminalia chebula* against different microorganisms (A) *Bacillus amyloliquefaciens*, (B) *Escherichia coli*, (C) *Salmonella enterica* ser. Typhi, and (D) *Aspergillus fumigatus*.
Table 1. Antimicrobial activity of *Terminalia chebula* fruit

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Name of Solvent</th>
<th>Diameter of zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Terminalia chebula</em> fruit</td>
<td>Acetone</td>
<td>B. amyloliquefaciens 30</td>
</tr>
<tr>
<td></td>
<td>Aqueous (cold)</td>
<td>B. amyloliquefaciens 25</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>B. amyloliquefaciens 30</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>B. amyloliquefaciens 30</td>
</tr>
<tr>
<td></td>
<td>Tetracycline (30 mcg)</td>
<td>B. amyloliquefaciens 21</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole (50 mcg)</td>
<td>B. amyloliquefaciens -</td>
</tr>
</tbody>
</table>

Note: -: No growth.

The methanolic extract was found to be most effective against all the test strains which exhibit zone of inhibition ranging from 25 mm to 30 mm followed by acetone, aqueous, ethanol and hot water. Aqueous and methanolic extracts showed the highest zone of inhibition of 30 mm against *Aspergillus fumigatus*. The antimicrobial activity of *Terminalia chebula* fruit extracts may be due to the presence of flavanoid, poly-urinoides, saponin, and tannin. Not a single research paper has been found for the antimicrobial activity of *Terminalia chebula* fruit against *B. amyloliquefaciens*, *S. epidermidis*, *S. enterica* ser. Typhi, *Aspergillus fumigatus* according to Prabhat et al. (2010), the maximum zone of inhibition against *S. aureus* i.e. 27 mm, dental carries pathogen such as *S. mutans* (23 mm), *L. acidophilus* (24 mm), *S. salivarius* and *C. albicans* (26 mm) were observed in methanolic extract of *Terminalia chebula* fruit. According to Sangeetha et al. (2012) the ethanol extract of *Terminalia chebula* fruit and seed was strongly inhibited to *S. aureus*, forming large zone of inhibition i.e. 20-26 mm in agar well diffusion and 14-24 mm in agar disc diffusion technique whereas it showed less activity against *C. albicans* as it formed 8-18 mm in agar well diffusion and 6-16 mm in agar disc diffusion technique. On the basis of literature, tetracycline showed the highest zone of inhibition against *Staphylococcus epidermidis* (34 mm) followed by *Bacillus amyloliquefaciens* (21 mm), *Escherichia coli* (20 mm) and *Salmonella typhi* (25 mm). Ketoconazole showed zone of inhibition against *Aspergillus fumigatus* (10 mm). Sharma et al. (2011) reported that ampicillin showed the highest zone of inhibition against *S. aureus* (22 mm) followed by *P. valgaris* (42 mm) and *E. coli* (16 mm) and the antifungal positive control fluconazole showed zone of inhibition against *A. niger* (32 mm) and *C. albicans* (32 mm). On the basis of maximum zone of inhibition the methanolic extract was further subjected to minimum inhibitory concentration by macro dilution agar method against tested microorganisms, and the results are shown in Fig. 2 and Table 2.
Figure 2. Minimum inhibitory concentration of methanolic extract of *Terminalia chebula* fruit against different microorganisms (A) *Bacillus amyloliquefaciens*, (B) *Escherichia coli*, (C) *Salmonella enterica* ser. Typhi, and (D) *Aspergillus fumigates*.

Table 2. Minimum inhibitory concentration of methanolic extract of *Terminalia chebula* fruit against different tested microorganisms.

<table>
<thead>
<tr>
<th>Name of microorganisms</th>
<th>Concentration of methanolic extract (mg/mL)</th>
<th>MIC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
<td>0.75</td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. enterica</em> ser. Typhi</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The MIC of methanol extract of *Terminalia chebula* fruit ranged from 0.125 mg/mL to 0.25 mg/mL. The MIC value was found to be 0.25 mg/mL against *Bacillus amyloliquefaciens* (Gram positive) and *Aspergillus fumigatus* (mould). The MIC was found less than 0.125 mg/mL against rest of the three bacteria such as *S. epidermidis*, *E. coli*, and *S. enterica* ser. Typhi. There is a further need to evaluate the exact value of MIC. The clinical pathogen *E. coli* showed a MIC value of 6.25 mg/mL (Chattopadhyay et al., 2007). The MIC of ethanol extract of *Terminalia chebula* fruit against *S. aureus* was recorded as 600 μg/mL (Sangeetha et al., 2012). Phytochemical analysis of most promising methanolic extract showed the presence of flavanoid, poly-urinoides, saponin, tannin and absence of alkaloid, keller-killani test and steroid (Table 3).
The phytochemical analysis of these six plants (*Achyranthes aspera*, *Acacia catechu*, *A. arabica*, *G. glabra*, *Mimusops elengi*, and *Terminalia chebula*) showed the presence of following biologically active plant constituents glycosides, flavonoids, alkaloids, and saponins were detected in *A. aspera*, *A. arabica* and *Mimusops elengi*. Tannins were detected in all extracts, saponins were not found in *Terminalia chebula*, *A. catechu* and *G. glabra*. Phytochemical screening of ethanolic extracts of *T. avicennioides* shows the presence of glycosides, saponins, tannins, alkaloids, steroids and phenol (Mann et al., 2008). In the present study, Gram positive bacteria were found to be more sensitive than Gram negative bacteria. This may be due to the fact that the cell wall in Gram-positive bacteria consists of a single layer which make their cell wall permeable to antimicrobial agents. Where as Gram-negative bacteria have an outer phosphor-lipid membrane with structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents. Methanolic extract of *Termialia chebula* fruit are more susceptible to microorganisms as compare to rest of the extracts and their MIC value ranged from 0.125 mg/mL to 0.25 mg/mL. Two possibilities that may account for the higher antibacterial activity of alcoholic extracts are the nature of biological active components (alkaloids, flavonoids, essential oil, tarpenoids, tannins, etc.), which may be enhanced in the presence of alcohol; and the stronger extraction capacity of alcohol that may have yielded a greater number of active constituents responsible for antibacterial activity (Ghosh et al., 2008). The methanolic extract is highly effective against all pathogens because more organic compounds were leached in this solvent. The inhibitory effect of the extracts may be attributed to the presence of bioactive metabolites. Several reports have shown that bioactive compounds isolated from plant extract have growth inhibitory effect on pathogenic strains. Plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have antimicrobial properties *in vitro*. Tannin is a general descriptive name for a group of polymeric phenolic substances. Many physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and a wide range of anti-infective actions have been assigned to tannins. Their mode of antimicrobial action may be related to their ability to inactivate microbial enzymes and transport proteins. Some of these metabolites particularly some flavonoids (that are absent) were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Yusha’u et al., 2008). In addition, some alkaloids and tannins are well documented for antimicrobial activity (Sign and Bhat, 2003). Sato et al. (1997) reported that a fruit ethanol extract of *Terminalia chebula* exhibited antibacterial activity

### Table 3. Phytochemical analysis of *Terminalia chebula* fruit methanolic extract

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Solvent</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Keller-killani Test</th>
<th>Poly-urinoides</th>
<th>Saponin</th>
<th>Steroid</th>
<th>Tannin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Terminalia chebula</em> Fruit</td>
<td>Methanolic extract</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The inhibitory effect of the extracts may be attributed to the presence of bioactive metabolites. Several reports have shown that bioactive compounds isolated from plant extract have growth inhibitory effect on pathogenic strains. Plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have antimicrobial properties *in vitro*. Tannin is a general descriptive name for a group of polymeric phenolic substances. Many physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and a wide range of anti-infective actions have been assigned to tannins. Their mode of antimicrobial action may be related to their ability to inactivate microbial enzymes and transport proteins. Some of these metabolites particularly some flavonoids (that are absent) were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Yusha’u et al., 2008). In addition, some alkaloids and tannins are well documented for antimicrobial activity (Sign and Bhat, 2003). Sato et al. (1997) reported that a fruit ethanol extract of *Terminalia chebula* exhibited antibacterial activity
against S. aureus (MRSA) and the compounds responsible for this activity were gallic acid and its ethyl ester.

4. Conclusions

The findings of the present investigation validate their traditional use and suggests that methanolic extract of *Terminalia chebula* fruit has better efficacy and can be a source for natural antimicrobial agent.

Acknowledgements

The authors are thankful to Director and Management of Ambala College of Engineering and Applied Research, Ambala for providing research Lab facilities and encourage us to complete this research work.

References


Copyright © 2013 by Modern Scientific Press Company, Florida, USA


