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Antibacterial activity of *Terminalia chebula* fruit extract

Kannan P.[1], Ramadevi S.R.[2] and Waheeta Hopper[3*]

Centre for Biotechnology, SPIC Science Foundation, 88, Mount road, Guindy, Chennai, India - 600 032
[1]Department of Environmental Engineering, Konkuk University, 1- Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea.
[2]Biotherapeutics Group, Avestha Gengraine Technologies (P) Ltd, Bangalore, India - 560 066.
[3]Department of Biotechnology, SRM University, Kattankulathur, India - 603 203.

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An ethanol extract of *Terminalia chebula* fruit was studied for its antibacterial activity against clinically important standard reference bacterial strains. The antimicrobial susceptibility was screened using the disc diffusion method and the minimum inhibitory concentration (MIC) was determined using the broth microdilution method. The results showed that it was active against both gram-positive and gram-negative bacteria. The *T. chebula* fruit extract was highly effective against *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853. The MIC was determined as 1 mg/ml for *S. typhi*. These results indicate that the *T. chebula* dry fruit possesses a potential broad spectrum of antimicrobial activity and a search for the active compound is needed.

Key words: *Terminalia chebula*, fruit, antibacterial susceptibility test, disc diffusion, broth microdilution, MIC.

INTRODUCTION

Higher plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is known as secondary metabolites. Some of these secondary metabolites are produced for self-defense (Evans et al., 1986) . Over the last 20 years, a large number of secondary metabolites from different plant species have been evaluated for their antimicrobial activity. The demand on plant-based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non - narcotic, easily biodegradable, pose minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices.

Terminalia chebula Retz. belongs to the family "Combretaceae", commonly known as black myrobalan. *T. chebula* is a medium- to large-sized tree distributed throughout tropical and sub-tropical Asia, including China and Tibet. This tree is found in the forests of northern India, Uttar Pradesh and Bengal, and is common in Tamil Nadu, Karnataka and southern Maharastra. The traditional Indian systems of Ayurveda and Siddha medicines support the importance of medicinal plants to treat diseases (Beusher et al., 1994). *T. chebula* is routinely used as traditional medicine by tribals of Tamil Nadu to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections (Dash and Bhagwan, 1991). Plant fruits appear to have evolved complex antibiotic compounds to cure various diseases like cancer, cardiovascular, digestive and pathogenic bacteria. Antibacterial activity of *T. chebula* extracts against several bacterial strains have been reported (Malckzadeh et al., 2001; Kim et al., 2006; Chattopadhyay et al., 2007; Bag et al., 2009). It is effective in inhibiting *Helicobactor pylori* (Malckzadeh et al., 2001), *Xanthomonas campestris* pv. *citri* (Afzalakhtar et al., 1997) and *Salmonella typhi* (Rani and Khullar, 2004). An aqueous extract of *T. chebula* fruit exhibits antifungal activity against a number of dermatophytes and yeasts (Dutta et al., 1998). It possesses antiviral activity against Herpes simplex virus type-1 (HSV-1), Human immunode-

corresponding authors E-mail: kannan_microl@yahoo.com, waheetahop@yahoo.com.

ficiency virus -1 (HIV-1) and Cytomegalovirus (Yukawa et al., 1996). In view of these reported medicinal values, the present work was carried out to examine the antibacterial potential of an ethanol extract of *T. chebula* fruits against clinically important CLSI (Clinical and Laboratory Standards Institute) reference bacterial strains.

MATERIALS AND METHODS

Plant material and extraction

T. chebula fruits were collected from the Chengalpatu District, Tamilnadu, India. The plant species was confirmed by a botanist and a voucher specimen (CB/P286) was preserved at the Centre for Biotechnology, SPIC Science Foundation, Chennai. The bulk quantity of fruit pulp was shade-dried. This dried pulp (427 g) was soaked in ethanol for 24 h. The ethanol extract was dried at low temperature under reduced pressure in a rotary evaporator to obtain a residue of crude extract. This crude extract was dissolved in dimethyl sulfoxide (DMSO) and used for the antimicrobial study.

Microorganisms

The bacterial strains *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 14380) were CLSI (formerly NCCLS-National Committee for Clinical Laboratory Standards, 1999) standard reference strains used for antibacterial susceptibility tests. A local isolate, *S. typhi* (SSFP 4S), was also included in this study. The stock cultures were maintained in 20% glycerol at -70^{O}C for long-term preservation and working cultures were maintained in Nutrient agar (NA) slants at 4^{O}C.

Antibacterial screening

Antibacterial screening was carried out using the standard disc diffusion test (Bauer et al., 1966). Different concentrations of compounds (1 and 0.5 mg/disc) were incorporated in 6-mm-diameter sterile discs (Himedia, India) and dried. Six discs were placed on a 90-mm Mueller Hinton agar (MHA) plate (Himedia, India) seeded with test bacteria, including a streptomycin standard antibiotic disc. After overnight incubation at 37^{O}C, the agar plates were observed for zones of inhibition.

Broth microdilution method

The broth microdilution method was carried out in a 96-well microtiter plate to determine the minimum inhibitory concentration (MIC). The different concentrations of compounds (1, 0.5, 0.25 and 0.125 mg/ml) were diluted in Mueller Hinton broth and the final volume was maintained at 100 µl. The final concentration of DMSO was less than 1%. Five (5) µl of an overnight grown bacterial culture was added to the test medium to bring the final inoculum size to 1×10^5 cfu/ml (Kannan et al., 2006). The agar plates were incubated at 37^{O}C for 16 h and the absorbance was read at 600 nm. The percent growth inhibition was calculated by comparison with a control using the formula indicated below. The lowest concentration of the compound that inhibits the complete growth of the bacterium was determined as the MIC.

$$\% \text{ of growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Statistical analysis

Values are expressed as mean ± SE. Statistical significance was determined using one- way analysis of variance (ANOVA) and values with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

T. chebula is an important medicinal plant in Indian traditional medicine and it is most frequently used herb in Ayurveda. The dried fruit pulp was extracted with ethanol and the residue (1.09%; 4.67g) was recovered using a rotary evaporator. The tested bacterial strains showed different patterns of inhibition (Table 1). The extract showed a broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria. This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity (Ahmad et al., 1998). The broad spectrum of antibacterial activity was reported for *T. chebula* (Phadke and Kulkarni, 1989) and *T. arjuna* (Singh et al., 2008). The ethanol extract at a concentration of 1 mg/disc showed maximum inhibition against *S. epidermidis*, followed by *B. subtilis*. Gupta et al. (2002) reported that a *T. pallida* fruit methanolic extract showed maximum activity against gram -negative bac-teria, while that of *T. bellerica* showed the highest inhibi-tion zones against *P. aeruginosa* and *E. coli* (Ghosh et al., 2008). Two possibilities that may account for the hig-her antibacterial activity of alcoholic extracts are the nature of biological active components (alkaloids, flavo-noids, essential oil, tarpenoids, tannins, etc.), which may be enhanced in the presence of ethanol; and the stronger extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for anti-bacterial activity (Ghosh et al., 2008).

The ethanol extract was further subjected to the broth microdilution method to determine the MIC (Table 2). The maximum activity was observed against *S. typhi*, followed by *S. epidermidis* at a concentration of 1 mg/ml. This result is in agreement with the report of Phadke and Kulkarni (1989) and Rani and Khullar (2004) studied in *T. chebula* and *T. arjuna* leaves, respectively. In this study, 90% growth inhibition (IC_{90}) was found against *S. epidermidis* and nearly 80% growth inhibition was observed against *P. aeruginosa* at a concentration of 1 mg/ml (Figure 1). This present study showed antibacterial activity at a low concentration, whereas Ahmad et al. (1998) reported similar activity at a concentration of 200 mg/ml. It has been reported that the pure compound arjunctin from *T. arjuna* was responsible for activity against *S. epidermidis* (Singh et al., 2008).

About 70% growth inhibition (IC_{70}) of *B. subtilis* was found at a concentration of 0.25 mg/ml. The IC_{50} was also determined at 1 mg/ ml against *S. aureus* and *E. coli*. *T. arjuna* was found to have antibacterial activity against *B. subtilis* and *S. aureus* (Perumalsamy and Ignacimuthu,

Table 1. Antibacterial activity of *Terminalia chebula* fruit ethanol extract.

Organisms	Streptomycin (10 µg/disc)	Zone of inhibition(mm diameter)	
		1 mg/disc	0.5 mg/disc
Bacillus subtilis MTCC 441	13	11	15
Staphylococcus aureus ATCC 25923	12	10	13
Staphylococcus epidermidis MTCC 3615	-	12	16
Escherichia coli ATCC 25922	13	8	9
Salmonella typhi SSFP 4S	9	10	12
Pseudomonas aeruginosa ATCC 27853	13	9	13
Klebsiella pneumonia ATCC 14380	11	8	9

- no activity.

Table 2. Antibacterial activity of *Terminalia chebula* fruit ethanol extract using broth micro-dilution method.

Organisms	Control	Concentration (mg/ ml)			
		1	0.5	0.25	0.125
Bacillus subtilis MTCC 441	0.416 ± 0.01^{a}	NA	NA	0.115 ± 0.00^{c}	0.149 ± 0.00^{b}
Staphylococcus aureus ATCC 25923	0.250 ± 0.05^{a}	0.118 ± 0.03^{c}	0.125 ± 0.00^{bc}	0.182 ± 0.01^{abc}	0.193 ± 0.00^{ab}
Staphylococcus epidermidis MTCC 3615	0.196 ± 0.02^{a}	0.014 ± 0.00^{d}	0.063 ± 0.02^{c}	0.093 ± 0.01^{bc}	0.120 ± 0.02^{b}
Escherichia coli ATCC 25922	0.153 ± 0.01^{a}	0.078 ± 0.01^{c}	0.106 ± 0.0^{bc}	0.150 ± 0.01^{a}	0.127 ± 0.01^{ab}
Salmonella typhi SSFP 4S	0.158 ± 0.01^{a}	0^{e}	0.032 ± 0.01^{d}	0.080 ± 0.01^{b}	0.048 ± 0.00^{c}
Pseudomonas aeruginosa ATCC 27853	0.456 ± 0.01^{a}	0.012 ± 0.00^{e}	0.060 ± 0.01^{d}	0.239 ± 0.00^{c}	0.308 ± 0.01^{b}
Klebsiella pneumonia ATCC 14380	1.056 ± 0.00^{a}	0.641 ± 0.01^{e}	0.854 ± 0.01^{d}	0.929 ± 0.00^{c}	0.968 ± 0.00^{b}

Values are absorbance (at 600 nm) mean ± SE of triplicates.
Data were analyzed by one-way ANOVA and values followed by the same alphabet letter are not significantly different. NA, not available; ATCC, American Type Culture Collection; MTCC, Microbial Type Culture Collection; SSFP, SPIC Science Foundation-Patholab

2001). Sato et al. (1997) reported that a fruit ethanol extract of *T. chebula* Retz. Exhibited antibacterial activity against *S. aureus* (MRSA) and the compounds responsible for this activity were gallic acid and its ethyl ester. The clinical pathogen *E. coli* showed a MIC value of 6.25 mg/ml (Chattopadhyay et al., 2007), which is six times higher than the present study. Terpenoides from *T. avicennioides* showed antibacterial activity against *S. aureus, E. coli* and *P. aeruginosa* (Mann et al., 2009). Nearly 40% growth inhibition was observed in *K. pneumoniae* at a concentration of 1 mg/ml. This is in agreement with the report of Suguna et al. (2002).

In conclusion, the *T. chebula* fruit ethanol extract sho-wed a broad spectrum of activity against CLSI reference bacterial strains. It showed maximum activity against *S. typhi*, *S. epidermidis* and *B. subtilis*. These results support the beneficial effects of *T. chebula* fruit for its antibacterial or antiseptic capacities. However, further studies are warranted on the extract to identify the active antibacterial compounds.

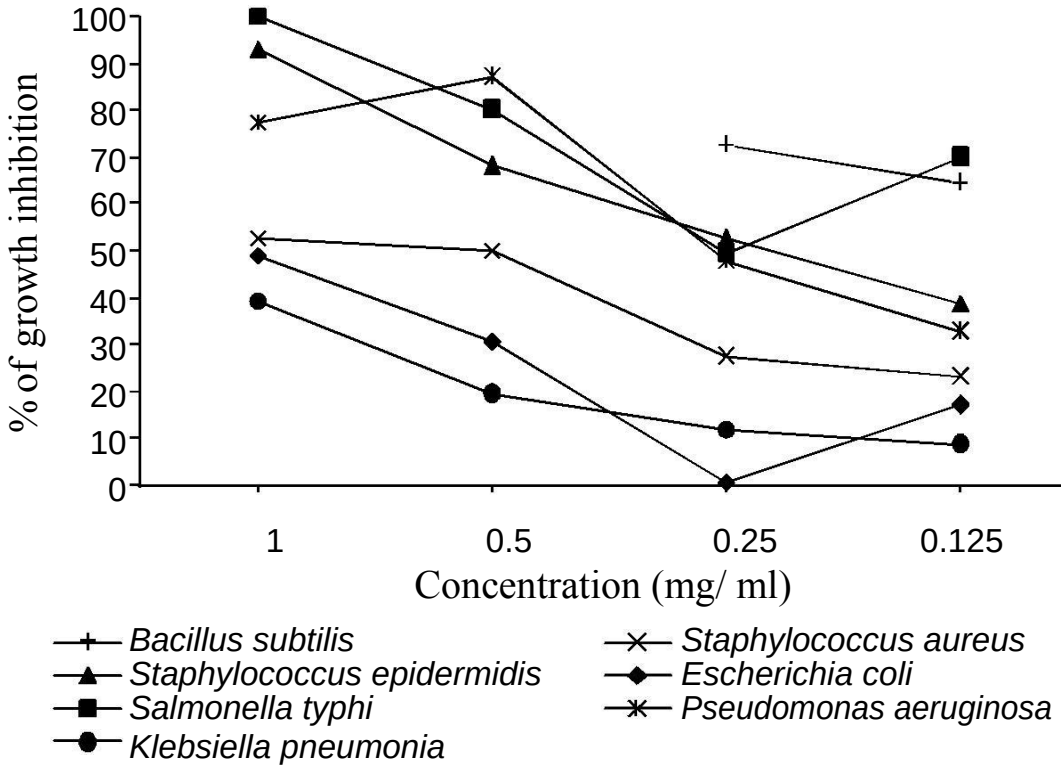

Figure 1. Percentage of growth inhibition of *Terminalia chebula* fruit ethanol extract against different bacterial strains.

REFERENCES

Afzalakhtar M, Rabber-Bhatti MH, Aslam M (1997). Antibacterial activity of plant Diffusate against *Xanthomonas compestris pv*. citri. Int. J. Pest Manage. 43(2):149-153.

Ahmad I, Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62(2):183-193.

Bag A, Bhattacharyya SK, Bharati P, Pal NK, Chattopadhyay RR (2009). Evaluation of antibacterial properties of Chebulic myrobalan (fruit of *Terminalia chebula* Retz.) extracts against methicillin resistant *Staphylococcus aureus* and trimethoprim-sulphamethoxazole resistant uropathogenic *Escherichia coli*. Afr. J. Plant Sci. 3(2):025-029.

Bauer AW, Kirby WMM, Sherris JC, Turk M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496.

Beusher N, Bodinet C, Neumann-Haefelin D, Marston A, Hostettmann K (1994). Antiviral activity of African medicinal plants. J. Ethnopharmacol. 42:101-109.

Chattopadhyay RR, Bhattacharyya SK, Medda C, Chanda S, Datta S, Pal NK (2007). Antibacterial activity of black myrobalan (Fruit of *Terminalia chebula* Retz.) against uropathogen *Escherichia coli*. Phcog. Rev. 11:212-215.

Dash B (1991). Materia Medica of Ayurveda. New Delhi: B. Jain Publishers, New Delhi. pp. 170-174.

Dutta BK, Rahman I, Das TK (1998). Antifungal activity of Indian plant extracts. Mycoses. 41(11-12): 535-536.

Evans JS, Pattison E, Morris F (1986). Antimicrobial agents from plant cell culture, In: secondary metabolites in plant cell culture. Edited by Morris P, Scraggs A, Stafford A, Fowler M (Cambridge University, London). p.12.

Ghosh A, Das BK, Roy A, Mandal B, Chanda G (2008). Antibacterial activity of some medicinal plant extracts. J. Nat. Med. 62:259-262.

Gupta M, Mazumder UK, Manikandan L, Bhattacharya S, Haldar PK, Roy S (2002). Antibacterial activity of *Terminalia pallida.* Fitoterapia 73:165-167.

Kannan P, Shanmugavadivu B, Petchiammal C, Hopper W (2006). *In vitro* antimicrobial activity of *Wrightia tinctoria* leaf extracts against skin microorganisms. Acta Bot. Hung. 48(3-4):323-329.

Kim HG, Cho JH, Jeong EY, Lim JH, Lee SH (2006). Growth inhibitory activity of active component of *Terminalia chebula* fruits against intestinal bacteria. J. Food Prot. 69(9):2205-2209.

Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR (2001). Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobactor pyloli*. Int. J. Antimicrob. Agents. 18:85-88.

Mann A, Amupitan JO, Oyewale AO, Okogun JI, Ibrahim K (2009). Antibacterial activity of terpenoidal fractions from *Anogeissus leiocarpus* and *Terminalia avicennioides* against community acquired infections. Afr. J. Pharm. Pharmacol. 3(1): 22-25.

National Committee for Clinical Laboratory Standards [NCCLS], (1999). Document M31-A performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard. NCCLS, Villanova, p. 57.

Perumal *S*R, Ignacimuthu S (2001). Antibacterial Effects of the Bark of *Terminalia arjuna*: Justification of Folklore Beliefs. Pharm. Biol. 39(6): 417-420.

Phadke SA, Kulkarni SD (1989). Screening of in vitro antibacterial activity of *Terminalia chebula*, *Eclapta alba* and *Ocimum sanctum*. Indian J. Med. Sci. 43(5): 113-117.

Rani P, Khullar N (2004). Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi.* Phytother. Res. 18: 670-673.

Sato Y, Oketani H, Singyouchi K, Ohtsubo T, Kihara M, Shibata H,

Higuti T (1997). Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* Retz. against methicillin-resistant *Staphylococcus aureus*. Biol. Pharm. Bull. 4: 401-404.

Singh DV, Gupta MM, Santha KTR, Saikia D, Khanuja SPS (2008). Indian ocean dipole mode and tropical cyclone frequency. Current Science. 94(1):10.

Suguna L, Singh S, Sivakumar P, Sampath P, Chandrakasan G (2002). Influence of *Terminalia chebula* on dermal wound healing in rats. Phytother. Res. 16:227–231.

Yukawa TA, Kurokawa M, Sato H, Yoshida Y, Kageyama S, Hasegawa T, Namba T, Imakita M, Hozumi T, Shiraki K (1996). Prophylactic treatment of cytomegalovirus infection with traditional herbs. Antiviral. Res. 32(2): 63-70.