Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
4(4):723-726
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

Evaluation of antibacterial activity of fruit extracts of *Terminalia chebula* against Human Pathogenic Bacteria

Kadudula Prasuna[1] and Yalavarthy Prameela Devi[2]*

[1,2]*Department of Zoology, Kakatiya University, Warangal. Telangana, INDIA*

ABSTRACT

Terminalia chebula, belongs to a family Combretaceae, is a native of South Asia. The fruit of this plant is one among triphala of ayurveda. This tribal medicine is used to treat for various diseases like throat infections, cellulitis, ear infections and oral infections which are caused by microorganisms. Antibacterial activities of *Terminalia chebula* extracts against several bacterial strains have been reported. The objective of the present study was to evaluate the possible antimicrobial potential of *Terminalia chebula* fruit extracts in acetone, ethanol, methanol and water against five streptococci bacteria. Five bacteria i.e., *Escherichia coli, Pseudomonas aeruginosa, Shigella boydii, Staphylococcus aureus and Streptococcus faecalis* were tested using blood agar as the medium by agar well diffusion and microdilution method. Only methanol extracts showed antibacterial activity against all five species. The highest activity was shown by the methanol extract with a mean diameter of inhibition zone being 14 mm and a minimum inhibitory concentration (MIC) of 1 mg/ml against *E. coli* followed by *S. boydii* and *S. fascalis*. These promising findings suggest the presence of antibacterial activity in the tested plant material, exhibited by its bioactive compounds and serving them as an alternative antimicrobial agent against the tested microorganisms.

Keywords: Antibacterial activity, *Terminalia chebula, S. boydii, S. fascalis, S. aureus, S. faecalis, P. aeruginosa*

INTRODUCTION

Annually fourteen million deaths are occurring due to infectious diseases (Walsh, 2003) and amongst them, bacterial infections are a major threat (Westh et al, 2004) and the only solution to this problem is use of antibiotics or chemicals. However, the increasing failure of chemotherapy and antibiotic resistance exhibited by bacterial pathogens has prompted researchers for screening of plants for their antimicrobial activity (Scazzocchio et al, 2001). Thus, there is an urgent need to discover new antimicrobials for new and re-emerging bacterial diseases.

How to Site This Article:
Kadudula Prasuna and Yalavarthy Prameela Devi, (2016). Evaluation of antibacterial activity of fruit extracts of Terminalia chebula against Human Pathogenic. *Biolife*. 4(4), pp 723-726.

DOI: 10.5281/zenodo.7350926
Received: 6 October 2016;
Accepted; 29 November 2016;
Available online : 6 December 2016

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). In view of these reported medicinal values, the present work

was carried out to examine the antibacterial potential of different solvent extracts of *T. chebula* fruits against clinically important human bacterial strains.

MATERIALS AND METHODS

Plant material and extraction

T. chebula fruits were collected from the Warangal District, Telangana State, India. The plant species was confirmed by a botanist and a voucher specimen was preserved at the Environmental Research Laboratory, Department of Zoology, Kakatiya University, Warangal. The bulk quantity of fruit pulp was shade-dried. This dried pulp (500 g) was soaked in different solvents (Acetone, Ethanol, Ethyl acetate and Methanol) for 36 h separately. The all extracts were dried at low temperature under reduced pressure in a rotary evaporator to obtain a residue of crude extracts. These crude extracts were dissolved in dimethyl sulfoxide (DMSO) and used for the antimicrobial study.

Bacterial Cultures:

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Staphylococcus aureus* and *Streptococcus faecalis* were obtained from the Department of Microbiology, Kakatiya University, Telangana State, India. All the test strains were maintained on nutrient agar slopes (Hi-Media) and were sub-cultured once in every two-week. These bacteria served as test pathogens for antibacterial activity assay.

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 × 105 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.

% of growth inhibition = Control – Test/Control X 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 different solvents and the residue was recovered using a rotary evaporator which is presented in Table-1. The amount obtained from ethaol ethyl acetate, acetone and methanol extracts are 1.25 gm (0.23%), 5.8 gm (1.06%), 7 gm (1.4%), and 9.070 gm (1.8 %) respectively. More yield obtained from methanol crude extract.

Table-1. Extractive values of different extracts of *T. chebula* fruits

S.No	Solvent	Initial Weight (gm)	Yield of the extract (in gm)	Percentage yield (%w/w)
1.	Ethanol (E)	500	1.25	0.230
2.	Ethyl Acetate (EA)	500	5.800	1.06
3.	Acetone (A)	500	7.000	1.4
4.	Methanol (M)	500	9.070	1.8

The tested bacterial strains showed different patterns of inhibition (Figure-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 (Sofowora, 1994). The broad spectrum of antibacterial activity was reported for *T. chebula* (Islam et al, 2002) and *T. arjuna* (Singh et al., 2008). The methanol extract at a concentration of 1 mg/disc showed maximum inhibition against *E. coli,* followed by *S. boydii,*and *S. fascalis.* The other solvent extracts not showed the antibacterial activity (data not shown). Kumar et al (2006) reported that a *T. pallida* fruit methanolic extract showed maximum activity against gram-negative bacteria, while that of *T. bellerica* showed the highest inhibition zones against *P. aeruginosa* and *E. coli* (Patra et al., 2009).

Figure-1. Mean values of Zone of inhibition against tested bacterial strains

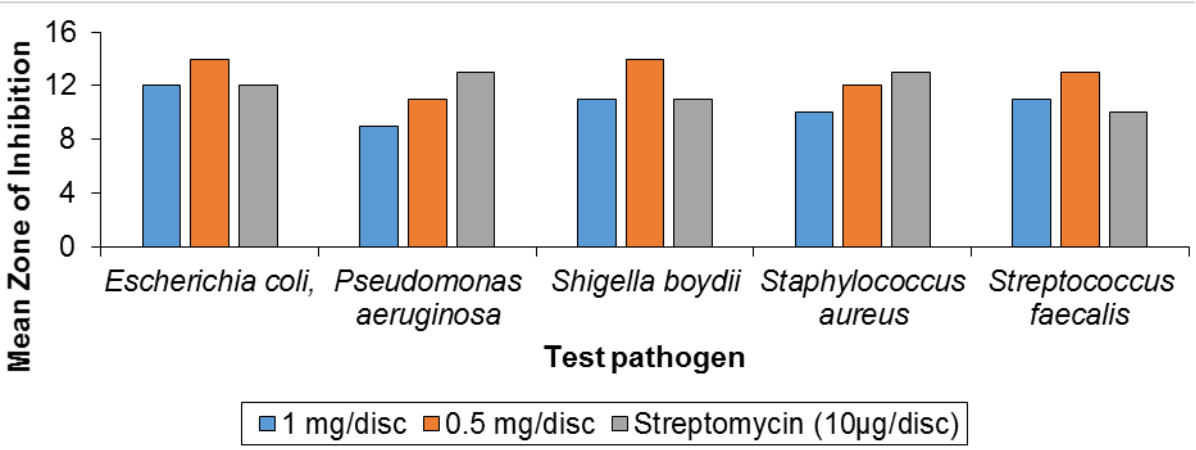

Table-2. Antibacterial activity of *Terminalia chebula* fruit ethanol extract using broth micro-dilution method.

Organisms	Control	Concentration (mg/ml)			
	0.125	0.25	0.50	1	Control
Escherichia coli	0.139±0.00b	0.114±0.001c	-	-	0.329±0.01*
Pseudomonas aeruginosa	0.182±0.00ab	0.172±0.01abc	0.114±0.01bc	0.109±0.02c	0.240±0.04*
Shigella boydii	0.122±0.02b	0.089±0.003bc	0.056±0.01c	0.014±0.01d	0.185±0.02*
Staphylococcus aureus	0.129±0.01ab	0.170±0.01b	0.109±0.01d	0.001±0.00*	0.152±0.01*
Streptococcus faecalis	0.987±0.04b	0.928±0.03c	0.834±0.01d	0.681±0.01*	1.046±0.01*

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 ethanol; and the stronger extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for antibacterial activity (Patra et al., 2008).

The methanol extract was further subjected to the broth microdilution method to determine the MIC (Table-3). 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 (IC90) 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; Lunavath Venkanna and Estari Mamidala, 2013).

About 70% growth inhibition (IC70) of *E. coli* was found at a concentration of 0.25 mg/ml. The IC50 was also determined at 1 mg/ ml against *Pseudomonas aeruginosa* and *Staphylococcus aureus Shigella boydii and Streptococcus faecalis.* Methanol and ethanol extract of Terminalia was found to have antibacterial activity against *E. coli* and *P. aeruginosa* and *S. aureus* (Perumalsamy and Ignacimuthu, 2001; Lalitha R & Mamidala E, 2013). Williamson et al. (2008) reported that a fruit ethanol extract of *T. chebula* Retz. Exhibited antibacterial activity against *S. aureus* 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 *Streptococcus faecalis* at a concentration of 1 mg/ml. This is in agreement with the report of Suguna et al. (2002).

CONCLUSION

In conclusion, the *T. chebula* fruit methanol extract showed a broad spectrum of activity against reference

bacterial strains. It showed maximum activity against *S. faecalis* and *P. aeruginosa*. 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.

Acknowledgement

Authors are thankful to express deep sense of gratitude to Department of Microbiology, Kakatiya University, Warangal for providing test organisms to carry out this work.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1]. 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.

[2]. 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.

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

[4]. 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.

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

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

[7]. Porika Raju and Estari Mamidala (2015). Anti-diabetic activity of compound isolated from *Physalis angulata* fruit extracts in alloxan induced diabetic rats. The Ame J Sci & Med Res, 2015,1(1); Pages 1 -6. doi:10.17812/ajsmr2015.11.1

[8]. Islam M, Azhar I, Usmanghani K, Ahmad A, Shahbuddin: Evaluation of antibacterial activity of *Bergenia ciliate*. Pak J Pharm Sci 15, 21–27 (2002)

[9]. 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.

[10]. 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.

[11]. Kumar MG, Jeyraaj IA, Jeyaraaj R, Loganathan P: 2006. Antimicrobial activity of aqueous extract of leaf and stem extract of *Santalum album*. Ancient Sci Life 25(3&4), 6–9.

[12]. Lalitha, R., & Mamidala, E. (2013). Invitro antimicrobial and antioxidant activity of acmella paniculata plant estracts. International Journal of Applied Biology and Pharmaceutical Technology, 4(1); 132-135.

[13]. Lunavath, Venkanna, and Estari Mamidala. "Preliminary phytochemical screening and antibacterial studies of the leaves of Eclipta alba (L)." International Journal of Pharmaceutical And Biology Sciences (2013): 380-384.

[14]. 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.

[15]. 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.

[16]. Patra JK, Panigrahi TK, Rath SK, Dhal NK, Thatoi H. Phytochemical screening and antimicrobial assessment of leaf extracts of *Exoecaria agallocha* L.: A mangal species of Bhitarkanika, Orissa, India. Adv Ind Nat Appl Sci 2009;3:241-6.

[17]. 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.

[18]. Scazzocchio F, Cometa MF, Tomassini L, Palmery M. Antibacterialactivity of *Hydrastis canadensis* extract and its major isolated alkaloids. Planta Med 2001;67:561-4.

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

[20]. Sofowora EA. Medical Plant and Traditional Medicine in Africa. Nigeria: University of Ife Press; 1994. p. 1-23.

[21]. Walsh C. Where will new antibiotics come from? Nat Rev Microbiol 2003;1:65-70.

[22]. Westh H, Zinn CS, Rosdahl VT. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microb Drug Resist 2004;10:169-76.

[23]. Williamson DA, Heffernan H, Sidjabat H, Roberts SA, Paterson DL, Smith M, 2008.: Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clin Infect Dis 1, 14–20.