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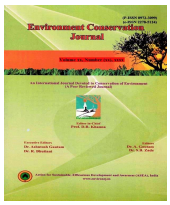

Antibacterial screening of *Terminalia chebula* Retz. against certain bacterial strains

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ABSTRACT

The aim of the study was to determine the antibacterial effectiveness of four different crude drug extract of *Terminalia chebula* (Badi Harad) extracted in methanol, ethanol acetone and aqueous solvent against certain food borne microorganisms i.e. *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio cholerae, Bacillus cereus,* and *Bacillus subtilis.* Methanolic extract had the strongest antibacterial effect, followed by acetone, ethanol, and aqueous extract. Maximum antibacterial activity obtained at 100% crude drug extract in methanolic was recorded against *E. coli* (25.6 mm) whereas in acetonic extract highest inhibition at 100% conc. was visualized against *S. typhi* and *V. cholerae* (22 mm).All four solvent crude extract crudes showed varient response in term of inhibition zone against all test organisms. Phytochemical analysis by standard methodology as well as by HPLC of the extracted crude samples showed presence of various phytoconstituents.

Introduction

Medicinal agents have been produced by natural resources since antiquity, and an incredible number of modern drugs have been discovered from these sources, especially those of plant origin, many of which were based on their use in traditional medicine. (Cowan, 1999). Viral infections and other infectious disorders have showed considerable potential for phytomedicines made from plants. About 25% of all medications are thought to be obtained either directly or indirectly from higher plants (Wickramsinghe, 2006). Eighty percent of the world's population, or about four billion people, use herbal remedies (Mukherjee, 2002; Bodeker *et al*., 2005; Wickramsinghe, 2006). Therapeutic properties of the plants are based on their metabolites which are stored in different parts of plant with their properties.The primary cause of death in humans is infectious disease. It is estimated that infectious diseases account for about 50% of fatalities (Iwu *et al.,* 1999). Antibiotics were first discovered in the 20th century provided a new line of treatment for controlling the infectious disease.Credit goes to Sir Alexander Fleming (1929), who discovered and introduced the first antibiotic to the world i.e. Penicillin. The treatment of infectious disease with antibiotics has been successfully done, but the scientists are forced to discover/launch the new antibiotics because of the failure or less effectiveness against the disease which were very effective earlier. Due to indiscriminate use of these antibiotics, various pathogenic microorganisms have gained resistance to these antibiotics. The issue of antimicrobial agent (antibiotic) resistance has gained importance on a global scale. This has generated a new generation of pathogens i.e. multi-drug resistant pathogens. Infectious illness management and treatment have been hampered by these multi-drug resistant pathogens. Due to the recurring occurrence of drug resistance in human diseases against currently prescribed antibiotics, a quest for novel antimicrobial chemicals from other sources, including plants, has become imperative. Since, the plants posses a large number of chemical components within them to protect themselves against a large number of pathogens. In addition, a

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variety of human ailments have been treated using plants from the dawn of humanity. There is a wealth of knowledge on the utilization of plants as antibacterial agents to combat human diseases. Large number of workers are carrying out researches on different aspects of plant based molecules. But most important aspect in this area is the study of antimicrobial activity of raw drug plant material extract or grinded powdered crude drug extract against human pathogenic organisms which provides a basic platform for further investigations and different aspects including enhancement of treatment of range of human diseases described in ancient text. Therefore this aspect becomes more significant. Large number of the workers are actively engaged in this area and voluminous reports are regularly coming but some of the important contribution are being quoted here. Several workers reported antimicrobial activity of different medicinal plants (Dwivedi *et al*., 2012; Chopra *et al.,* 2013; Adebayo *et al.,* 2014; Asimuddin *et al*., 2017; Lee and Hwang, 2021; Nam and Hwang, 2021).Due to its excellent healing properties, *Terminalia chebula* Retz., also known as the "King of Medicine" in Tibet, consistently ranks at the top of the "Ayurvedic Materia Medica" list. (Bag *etal.,* 2009). In Asia's tropical and subtropical regions, which include China and Tibet, the plant is widely dispersed. *T. chebula* contains a variety of phytoconstituents, including as tannins, flavonoids, sterols, amino acids, fructose, resins, etc. It is a top-ranked plant for treating gout, gouty arthritis, bleeding piles, sore throats, and asthma in the Ayurvedic Materia Medica (Aneja and Joshi, 2009). The rejuvenating properties of haritaki include laxative, astringent, anthelmintic, nervine, expectorant, tonic, carminative, and stimulant of appetite. The herb is used to make "Triphala," a laxative intended to treat persistent constipation. Triphala is the most adaptable herbal remedy and is recommended for candida infection and cardiotonic effects (Kaur *et al.,* 2005). It is recommended for those who have leprosy (which includes skin issues), anaemia, narcosis, piles, chronic intermittent fever, heart disease, diarrhoea, anorexia, cough, excessive mucus secretion, and a number of other complaints and symptoms. The goal of the current study was to study the antibacterial properties of *T. chebula* fruit against gram positive and gram negative bacterial strains.

Material and Methods

Collection of plant material and soxhlet extraction procedure:The fruits of *Terminalia chebula* were collected locally. The fruits were cleaned with running tap water, then sterilised distilled water, and thendried.. After proper drying fruits of *T. chebula* (Badi harad) were grinded into coarse powder. The coarse powder was extracted in soxhlet extractor for 24 hrs using different extractants methanol, ethanol, acetone, and water The solution was then filtered through filter paper (Whatman No.1.). The resulting extract solution was referred to as stock solution (100 percent concentrated drug solution). The 100% concentrated solution was subsequently diluted with distilled water to produce 75%, 50%, and 25% concentrations.

Test organisms: Pure cultures of *Vibrio cholerae* (MTCC 3904), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 6728), *Salmonella typhi* (MTCC 3216), and *Staphylococcus aureus* (MTCC 7443) were obtained from the Microbial Type Culture Collection of the Institute of Microbial Technology (IMTECH) Chandigarh and *Escherichia coli* was obtained from from SGPGI Lucknow.

Antibacterial activity testing: To evaluate the effectiveness of plant extracts against bacterial strains, the agar well diffusion method was employed (Bell and Grundy, 1968). To 100 ml of nutritional agar medium, 2 ml of bacterial suspension was added. Nutrient agar medium was substituted with Luria bertani media for *V. cholerae*. The flask was gently swirled to ensure that the test organisms were distributed evenly. After that, sterile petri plates were filled with the inoculated culture media and allowed to solidify. A sterile cork borer with a 6mm diameter was used to create 5 wells in the set of each petriplate, 4 of which were located on the periphery and 1 in the centre. Four peripheral wells received 0.1ml (100µl) solutions of plant extract at 100%, 75%, 50%, and 25% of the various concentrations. A 0.1 ml solution of the control was poured into the centre wells. Methanol, ethanol, acetone, and sterilised distilled water were employed as controls for methanolic, ethanolic, acetonic, and aqueous

extract. Each Sample was assayed in triplicate and value was observed and recorded.

Phytochemical Analysis: The plant extracts were underwent qualitative screening to determine the different plant constituents as per standard procedures (Kokate *et al.,* 2007; Khandelwal, 2004).

Detection of alkaloids: One drop of Mayer‟s reagent was added to the extracts. The formation of precipitation with color change was observed.

Detection of Flavonoids: Add a few drops of concentrated H_2SO_4 producing deep yellow coloured solution indicated the presence of flavones and flavanols.

Detection of Steroids: Libermann-Burchard Test:- The extract was heated and then cooled after being treated with a few drops of acetic anhydride. Sulphuric acid was added from the side of the test tube, and a brown ring developed at the junction of two layers.

Detection of Saponin: Because they resemble soap, they are referred to as saponins. Separately, 1 ml of the extract was diluted to 20 ml with distilled water and stirred for 15 minutes in a graduated cylinder. A centimeter-thick layer of foam signify saponins.

Detection of Tanins: Ferric Chloride Test:- A 5% ferric chloride solution in water or ethanol was used to treat the extract. When many drops of solution are added to an extract, a blue-black or blue-green colour results, signifying the presence of tannins.

Detection of phytochemicals by HPLC

HPLC analysis of *T. chebula* (ethanolic and acetonic extracts) were carried out in order to detect the presence of phytoconstituents.

Results and Discussion

Result of antibacterial potency/efficacy of different extracts of *Terminaliachebula* based on four different solvent extraction i.e. methanol, ethanol, acetone and water against certain Gram-positive bacterial strains *B.subtilis*, *B.cereus, S.aureus* and Gram- negative bacterial strains *E.coli, S.typhi, V.cholerae* have been presented in table-1 and 2, Photoplate 1. Results of phytochemical screening of most prominent plant extracts of *T. chebula* have been depicted in table-3. Results of HPLC analysis have been shown in table-4. A positive correlation between drug concentration and drug potency has been found against all the test organisms in all four extracts. With the dilution of a 100% concentrated extract, the drug's effectiveness in terms of the inhibitory zone decreased.

Table 1: Antibacterial activity of methanolic and ethanolic extract of *T. chebula*

Extract Type	Test organism	Effective Zone of Inhibition ±SD (mm)			
		Extract concentration			
		100%	75%	50%	25%
Methanol	*Salmonella typhi*	23.0±0.00	20.6±0.57	16.3±0.57	14.0±0.00
	Escherichia coli	25.6±0.57	23.3±0.57	21.0±0.00	19.3±0.57
	Vibrio cholerae	23.0±1	21.3±0.57	18.0±0.00	14.0±0.00
	Staphylococcus aureus	24.0±1.00	22.3±0.57	20.0±1.00	17.6±0.57
	Bacillus cereus	18.6±0.57	16.6±0.57	15.3±0.57	13.6±0.57
	Bacillus subtilis	21.3±0.57	19.3±0.57	16.6±0.57	14.3±0.57
Ethanol	*Salmonella typhi*	19.0±1	16.0±0.00	14.0±1.00	11.6±0.57
	Escherichia coli	20.0±1	19.0±0.00	17.0±0.00	14.0±1.00
	Vibrio cholerae	21.6±0.57	19.0±0.00	16.0±1.00	14.0±0.00
	Staphylococcus aureus	20.6±0.57	18.0±1.00	16.0±0.00	13.0±1.00
	Bacillus cereus	20.3±0.57	17.3±0.57	16.3±0.57	13.6±0.57
	Bacillus subtilis	20.6±0.57	18.3±0.57	16.6±0.57	14.3±0.57
control		-	-	-	-

***Effective inhibition zone= Average value of inhibition zone of three replicates-well size(-)= No activity**

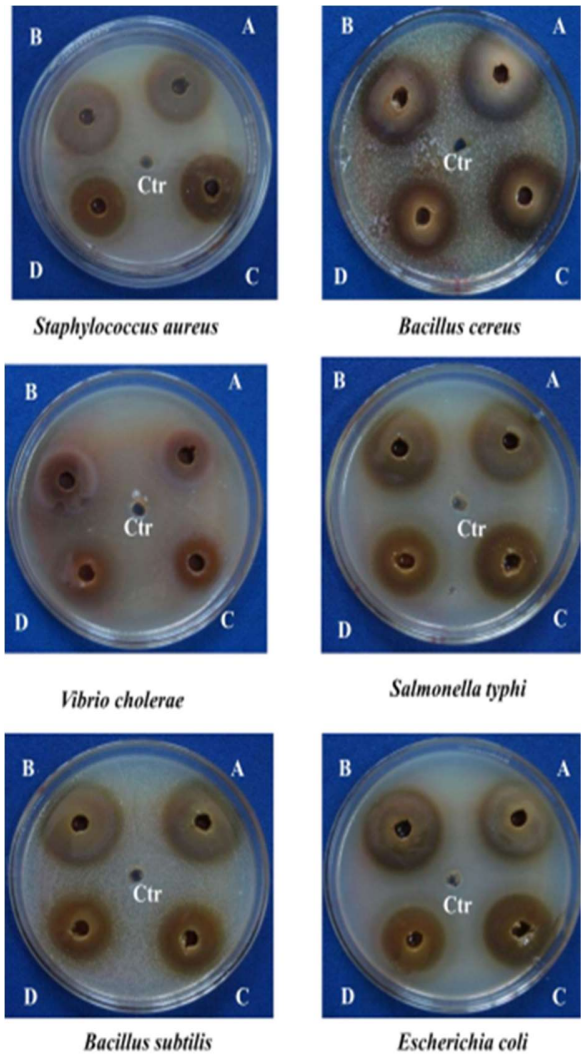
Methanolic extract of *T. chebula* was found more effective followed by acetone, ethanol and aqueous extracts. Maximum inhibitory activity in methanolic extract at 100% conc. was recorded against against *E. coli* followed by *S. aureus, S. typhi, V.cholerae, B. subtilis* and *B. cereus* whereas ethanolic extract revealed maximum activity against *V. cholerae* followed by *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli* and *S. typhi*.

Table 2: Antibacterial activity of acetonic and aqueous extract of *T. chebula*

***Effective inhibition zone= Average value of inhibition zone of three replicates-well size(-)= No activity**
SD= Standard deviation±of three replicates

Extract Type	Test organism	Effective Zone of Inhibition ±SD (mm) Extract concentration			
		100%	75%	50%	25%
Acetone	*Salmonella typhi*	22.0±1.0	20.0±0.00	17.0±1.00	15.0±1.0
	Escherichia coli	21.0±1.0	19.6±0.57	16.0±0.00	13.0±1.0
	Vibrio cholerae	22.0±0.0	19.0±1.00	16.6±0.57	14.0±0.0
	Staphylococcus aureus	21.0±1.0	19.0±1.00	16.6±0.57	14.0±1.0
	Bacillus cereus	17.0±0.0	16.0±1.00	14.0±0.00	11.0±1.0
	Bacillus subtilis	20.0±0.0	16.6±0.57	15.3±0.57	11.0±1.0
Aqueous	*Salmonella typhi*	12.0±1.0	10.3±0.57	9.0±0.00	5.0±1.0
	Escherichia coli	12.0±1.0	9.6±0.57	8.6±0.57	3.6±1.15
	Vibrio cholerae	10.3±1.1	8.6±0.57	7.6±0.57	3.0±0.0
	Staphylococcus aureus	12.6±0.5	10.3±0.57	7.0±1.73	4.0±1.0
	Bacillus cereus	16.0±0.5	14.3±0.57	11.6±0.57	5.6±0.57
	Bacillus subtilis	14.6±0.5	13.3±0.57	11.6±0.57	7.6±0.57
Control		-	-	-	-

Theacetonic crude extract of plant showed highest inhibitory activity against *S. typhi* and *V. cholerae* followed by *E. coli*, *S. aureus, B. subtilis* and *B. cereus* at 100% concentration. The aqueous plant extract at 100% conc. displayed maximum inhibitory activity against *B. cereus* followed by *B. subtilis*, *S. aureus*, *S. typhi*, *E. coli* and *V. cholerae.* The findings of earlier researchers support our findings. Sah *et al*. (2012) screened the antimicrobial potential of *T. chebula* fruit extracts, extracted in petroleum ether and methanol against *B. subtilis, S. aureus, E. coli, Klebsiella* spp. and *S. paratyphi* and found that methanolic extract was better than petroleum ether extracts. Similar to this, Jayalakshmi *et al.* (2011) assessed the antibacterial potential of *T. chebula* fruit extracts in petroleum ether, chloroform, ethyl acetate, and methanol against *E. coli, K. pneumonia, B. subtilis, B. cereus, S. typhi, E. aerogenes,* and *S. aureus*.In their research, petroleum ether, ethyl acetate, and methanolic extracts all shown the highest levels of inhibitory activity. Results of present study are also supported by findings of Kumar *et al*. (2013). The methanolic extract of *T. chebula* fruit was more effective than that of acetone, ethanol, water (cold and hot) extracts against certain bacterial species i.e. *B. amyloliquefaciens*, *S. epidermidis*, *E. coli*, *Salmonella enteric* ser *typhi* and *A. fumigates* which might be due to fact that more organic compounds were leached in methanol solvent as evidenced by Kumar *et al.*(2013).



Photoplate 1: Antibacterial activity of methanol extract of *T. chebula*against *S. aureus, B.cereus, V. cholera, S.typhi, B. subtilis* and *E. coli*.
Abb: A=100%, B=75%, C= 50%, D=25%

Results of preliminary phytochemical analysis has been depicted in table-3. Methanol extract of *T.chebula* were found to be rich in flavonoids, saponins and tannins but alkaloids, steroids and glycosides showed absence while in acetonic extract alkaloids, flavonoids, tannins, steroids and glycosides were analysed and detected but saponins showed absence. Gram-positive and Gram-negative bacteria are effectively inhibited by

methanolic extract, which may be attributed to the individual or combined effects of these phytochemicals. It is generally known that certain alkaloids and tannins have antibacterial properties (Sing and Bhat, 2003), probably due the mode of antimicrobial action of methanolic extract which is related with inactivation of microbial enzymes along with transport of proteins. In the present study, moderate activity in aqueous extracts has been observed against both Gram-negative and Gram-positive bacteria as also reported by Kumar *et al*. (2009).

Table 3: Phytochemical components present in different crude extracts of grinded test drug material of *T. chebula*

Extract type	Alkaloids	Flavonoids	Saponins	Tannins	Steroids
T. chebula (Methanol)	_	+	+	+	_
T. chebula (Acetone)	+	+	_	+	+

Table 4: HPLC analysis of *T. chebula*

Extract type	Quantification on percent dry weight basis of different compounds					
	Chlorogenic acid	Caffeic acid	Rutin	Myricitin	Quercetin	Kaempferol
T. chebula (Ethanol)	0.225977	0.155168	0.070655	0.124353	ND	ND
T. chebula (Acetone)	0.161361	0.202259	ND	ND	0.00601	ND

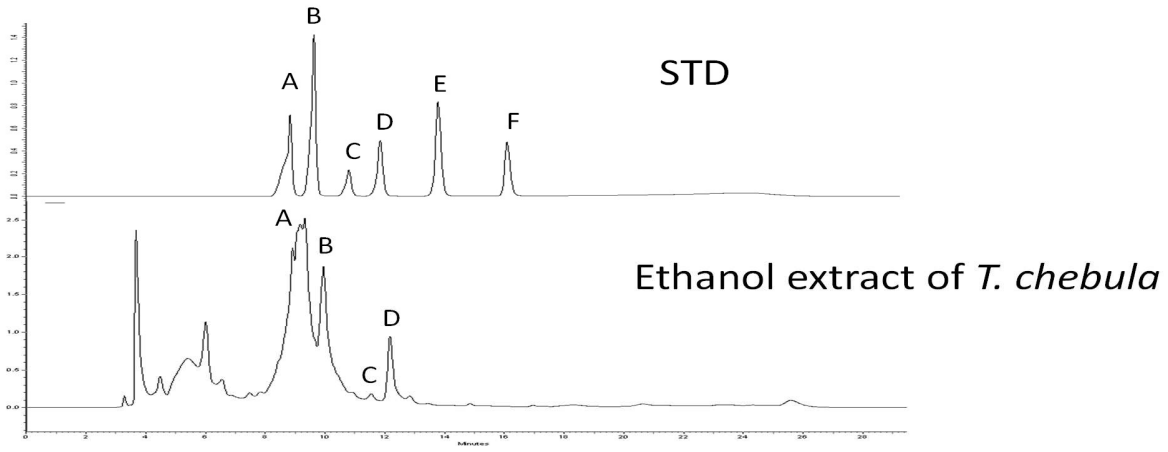


Figure 1: HPLC analysis of ethanol extract of *T. chebula*

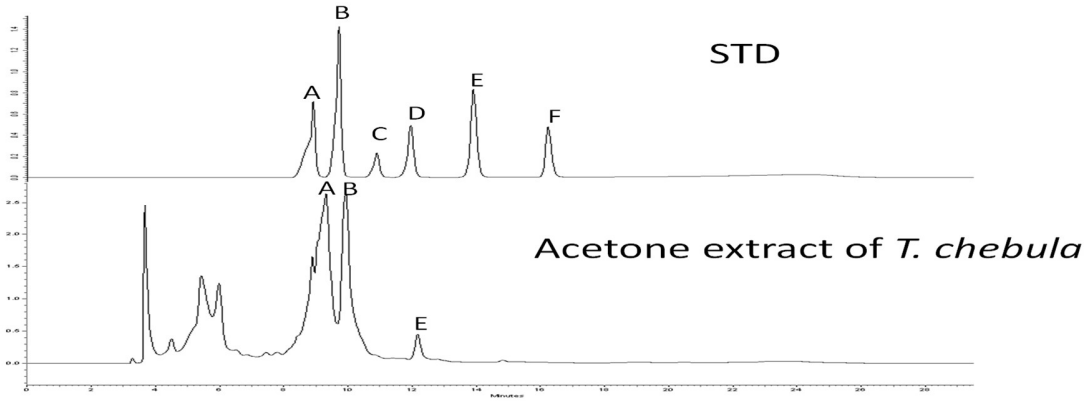


Figure 2: HPLC analysis of acetone extract of *T. chebula*

Description of peaks found:

A-Chlorogenic acid, B-Caffeic acid, C-Rutin, D-Myricitin, E-Quercetin, F-Kaempferol, STD: Standard

This may be due to the fact that variation in the extracted chemical component as well as very lesser quantity of the extracted component whichcould not effect the growth of test organisms. Lowered inhibitory effect of aqueous extract of *T. chebula* fruit is supported with the findings made by Ahmad *et al.* (1998). They evaluated three different extracts, extracted in alcohol, hexane and water and recorded lowest antibacterial activity in aqueous extract among all of them.

T. chebula ethanolic extract's HPLC examination revealed the presence of chlorogenic acid, caffeic acid, rutin, myricitin but quercitin and kaempferol were not detected (table-4) (Figure 1,). Similarly, the results of HPLC analysis of *T. chebula* acetonic extract (table-4; Figure-2) showed presence of three compounds i.e. chlorogenic acid, caffeic acid and quercitin. While rutin, myricitin and kaempferol were not found in acetone extract of *T. chebula.* These compounds have been found associated with antimicrobial activity. Observation made by HPLC analysis of *T. chebula* revealed that chlorogenic acid played a significant role in inhibition of different bacterial strains. Chlorogenic acid obtained from *Helichrysum* (Asteraceae) exhibited significant antimicrobial activity against *B. subtilis* and *B. cereus* (Albayrak *et al.,* 2010), whereas chlorogenic acid derived from carrot extracts showed higher antimicrobial activity against *L. monocytogenes* (Babic *et al.,* 1994). Infact chlorogenic acid derived from different variety of plants exhibits high antimicrobial activity against *E. coli* strains (Babic *et al.,* 1994; Albayrak *et al.,* 2010; Xia *et al.,* 2011a & b). Other constituents such as rutin, myricitin, quercitin have also found in *T. chebula*. The literature that is currently available demonstrated that these compounds play a substantial role in antibacterial action. Myricetin reported to have inhibitory activity against both Gram-positive and Gram-negative pathogenic bacteria (Tsai *et al.,* 2008; Freeman *et al.,* 2010). Askun *et al.* (2009 a & b) and Santas *et al.* (2010) reported antibacterial activity of quercitin against *B. subtilis* and *B. cereus* and *E. coli*.

Conclusion

With a broad range of pharmacological and therapeutic actions, *T. chebula* is one of the most popular plant world wide. This versatile plant's use as a medicine makes it a special source of several kinds of chemicals with distinct chemical structures. Our research indicated that the methanolic, acetonic *T. chebula* extracts have demonstrated positive antibacterial activity against all of the investigated bacterial pathogens, which accounts for its use in traditional systems of medicine. Consequently, *T. chebula* can be used as a source of natural antimicrobials as a complement to traditional medications.

Conflict of interest

The authors declare that they have no conflict of interest.

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