

# INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Original Article.....!!!

Received: 15-08-2012; Revised; Accepted: 18-08-2012

## ANTIMICROBIAL ACTIVITY OF *TERMINALIA CHEBULA* AGAINST URINARY TRACT PATHOGENS

R. Sangeetha, P. Nirmala\*

PG and Research Department of Microbiology, Mohamed Sathak College of Arts and Science,  
Sholinganallur, Chennai – 600 119, Tamilnadu, India

### Keywords:

*Terminalia chebula*,  
antimicrobial activity, Urinary  
Tract pathogens, MIC

### For Correspondence:

**Dr. P. Nirmala**

PG and Research Department of  
Microbiology, Mohamed Sathak  
College of Arts and Science,  
Sholinganallur, Chennai – 600  
119, Tamilnadu, India

### E-mail:

[nirmala\\_dineshkumar@yahoo.com](mailto:nirmala_dineshkumar@yahoo.com)

### ABSTRACT

The antimicrobial activity of the ethanolic extract of *Terminalia chebula* (dry Fruit and seed) was tested by both agar well diffusion and agar disc diffusion technique against Urinary Tract pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*). In both techniques, we found that ethanolic extracts of fruits showed maximum antibacterial activity against the Gram positive as well as Gram negative urinary tract pathogens than the ethanolic extracts of seeds. The fruit and seed extract of *T. chebula* was strongly inhibited to *S.aureus*, forming large zone of inhibition i.e. 20-26mm in agar well diffusion and 14-24mm in agar disc diffusion technique whereas it showed less activity against *C. albicans* as it formed 8-18mm in agar well diffusion and 6-16mm in agar disc diffusion technique. Based on the preliminary screening result, the ethanolic extracts of *T.chebula* fruit was found to be most potent antibacterial extract against *Staphylococcus aureus*. The MIC and MBC of the fruit extract against *S.aureus* was determined by tube dilution method. The MIC of fruit extract against *S.aureus* was recorded as 600µg/ml (no visible turbidity) whereas MBC was recorded as 800µg/ml. (no growth on plate). The phytochemical analysis of ethanol extract of *Terminalia chebula* fruit revealed the bioactive compounds which are responsible for the invitro antibacterial activity over the pathogens could be Tannins, Quinones, Phenols, Coumarins etc., These result indicate that the *T. chebula* dry fruit possesses a potential broad spectrum of antimicrobial activity.

## INTRODUCTION

The discovery of antibiotics more than 70 years ago initiated a period of drug innovation and implementation in human and animal health and agriculture. These discoveries were tempered and questioned in all cases by the emergence of resistant microbes<sup>1</sup>. The extensive use of the antibiotics to control diseases has led to the emergence of multidrug resistance<sup>2</sup>. It was warned by the World Health Organization that those multiple antibiotic-resistant pathogens would very likely bring the world back to the pre-antibiotic era. This clearly highlights the need for new antibacterial agents with fundamentally different modes of action than that of traditional antibiotics. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity<sup>3</sup>. For Centuries, plants have been used throughout the world as drugs and remedies for various diseases. These drugs serve as prototype to develop more effective and less toxic medicines. 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 environment hazards, have no adverse side-effects and are easily available at affordable prices. Hence, an attempt has been made to evaluate antibacterial activity of folklore medicinal plants. In India, medicinal plants form the backbone of several indigenous traditional systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds<sup>4</sup>. At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines<sup>5,6,7,8</sup>.

Urinary tract infections (UTI) are most common form of bacterial infections, affecting people throughout their lifespan. Multiple antimicrobial resistances among Gram negative organisms have been a long term and well recognized problem with urinary tract infections. Recently there has been a renewed interest in improving health and fitness through the use of natural products other than commercially available antibiotics or drugs.

*Terminalia chebula* is called the 'King of Medicine' in Tibet and is always listed at the top of the list in Ayurvedic Materia Medica due to its extraordinary power of healing<sup>9</sup>. *Terminalia chebula* Retz. belongs to the family "Combretaceae", commonly known as black myrobalan. *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. Different part of this plant has germinated substantial compounds to cure various diseases like cancer<sup>10</sup>, pathogenic bacteria<sup>11</sup>, diabetic<sup>12</sup>, and hepatoprotective<sup>13</sup> activity. Antibacterial activity of *T. chebula* extracts against several bacterial strains has been reported<sup>9, 14, 15</sup>. An aqueous extract of *T. chebula* fruit exhibits antifungal activity against a number of dermatophytes and yeasts. It possesses antiviral activity against Herpes simplex virus type-1 (HSV-1), immunodeficiency virus-1 (HIV-1) and Cytomegalovirus.

Phytochemical investigations of *Terminalia Chebula* have been reported on presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids<sup>16</sup>.

In view of these reported medicinal values, the present study was carried out to examine the antimicrobial potential of ethanolic extracts of *Terminalia Chebula* (fruit and seed) against urinary tract pathogens. Because the reports on antimicrobial activity of *Terminalia Chebula* fruit and seeds were scanty, particularly on urinary tract pathogens. The activity of the extract was finally quantitatively estimated in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. Phytochemical analysis of ethanolic extract of *T.chebula* fruit was also done.

## MATERIALS AND METHODS

All chemicals, media components and antibiotic impregnated discs used in this study were procured from Hi Media, Mumbai, India.

### Sample collection, isolation and identification of urinary tract pathogens

Clean catch mid stream urine samples from urinary tract infected persons were collected from the nearest hospital and transferred to the laboratory for processing. With the urine sample, a direct Gram staining was done and analyzed. For the isolation of microorganisms (bacteria and fungi), the urine sample was taken in a calibrated loop and streaked on Nutrient agar, MacConkey agar, blood agar and Sabouraud dextrose agar supplemented with streptomycin sulphate. Pure cultures of all morphologically suspected colonies (both bacteria and fungi) were maintained in slants and stored for further characterization. The bacterial pure culture obtained was further subjected to Gram staining, Motility and various biochemical tests (such as IMViC, Nitrate reduction, Urease, TSI, Carbohydrate Fermentation, Catalase and Coagulase) and streaked on selective media for the identification of each isolate<sup>17</sup>. Based on the colony

morphology and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology each bacterial isolate was identified. The yeast culture obtained was further subjected to Gram staining, Germ tube test, Carbohydrate assimilation test, Carbohydrate fermentation test and Urease test. Based on the microscopic and macroscopic observations and biochemical test, the yeast isolate was identified.

### **Collection of plant material and extraction**

The fresh matured dry fruits of *T.Chebula* (Figure 1) were collected from the Chengalpatu District, Tamilnadu, India. 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 the fruit pulp and seeds were separated. The bulk quantity of fruit pulp and the seeds were pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight containers and left in the refrigerator.

Figure 1. Dry fruits of *T.Chebula*



The coarsely powdered plant materials viz., fruits and seeds of *T. chebula* were successively use ethanol for its extraction. About 50g of seed powder and 150g of fruit powder was soaked in 150ml and 450ml of Ethanol (1:3 ratios) for 24h. Each preparation was filtered through a sterilized Whatman No.1 Filter paper and the filtered extract was concentrated to dryness in rotary vaccum evaporator below 40°C. The dried extracts thus obtained were exposed to UV rays (200-400nm) for 24 hours and checked frequently for sterility by streaking on Nutrient agar plates<sup>18</sup> and stored in labeled sterile bottles in a freezer at 4°C until further use.

This crude extract was dissolved in dimethyl sulfoxide (DMSO) and used for the antimicrobial study against selected pathogenic strains.

### **Determination of antimicrobial activity**

Muller Hinton Agar (MHA) was used as base medium for screening of antimicrobial activity and Muller Hinton broth (MHB) for preparation of inoculum.

A pure isolate of each microbe was subcultured on the recommended specific media. About 3-5 pure colonies of each organism were inoculated into MHB and the turbidity was adjusted to the McFarland Nephelometer standard 0.5 (approximately  $10^6$  cfu/ml) and used as an inoculum. For yeast, Sabaurouds dextrose broth (SDB) was used.

Screening of antimicrobial activity was performed by agar well diffusion technique<sup>19</sup> and standardized agar disc diffusion method<sup>20</sup>.

The stock solution of ethanolic extract was prepared as 200mg/ml of DMSO.

#### **Agar well diffusion technique**

0.1ml of standardized inoculum of each test organism was spread on to the specific media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. Different concentrations of ethanolic extract (fruit and seed) of *T. chebula* used for the study were 1mg (5 $\mu$ l), 2mg (10 $\mu$ l), 3mg (15 $\mu$ l) and 4mg (20 $\mu$ l). The dissolution of the organic extract was aided by 1% (v/v) DMSO, which did not affect the growth of microorganisms, in accordance with the control experiments. Different concentrations of fruit and seed extract was dispensed into the well separately and each plate was labeled with the test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24 hours and 25°C for 48 hours for bacteria and fungi, respectively. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well<sup>21</sup>. The experiments were performed in duplicate and repeated thrice.

#### **Agar disc diffusion technique**

The Muller Hinton agar plates and Sabaurouds dextrose agar plates were seeded with suspension ( $10^6$  cfu/ml) of the bacterial and fungal strains vice- versa. The empty sterilized Whatman No.1 filter paper disc (6mm) were impregnated with different concentrations 1mg (5 $\mu$ l), 2mg (10 $\mu$ l), 3mg (15 $\mu$ l) and 4mg (20 $\mu$ l) of ethanolic extract (fruit and seed) of *T. chebula* aided by 1% (v/v) DMSO, dried and placed aseptically on seeded plates with the help of a sterile forceps. The standard antibiotic disc (Ciprofloxacin) which was prepared in the same manner was used as positive control and DMSO was used as negative control. For yeast, Sabaurouds dextrose agar (SDA) was used and Fluconazole was used as positive control.

Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center. The agar plates were then incubated at 37°C for 24 hrs and 25°C for 48 hours for bacteria and fungi, respectively. The experiments were performed in duplicate and repeated thrice.

### **MIC and MBC**

The Minimum inhibitory concentration (MIC) of the fruit extract was determined by tube dilution techniques in Mueller-Hinton broth (Merck) according to NCCLS<sup>21</sup>. The range of concentration used was 1000µg –200µg/ml. 0.9ml of Mueller-Hinton broth (MHB) was taken in each of sterile and dry glass vials and 1.0ml of the respective extract concentration was dispensed into respective vials. 100µl of bacterial suspension of interest that was previously grown in nutrient broth were added to vial and incubated at 37°C for 24 hours. The lowest concentration that exhibited no visible growth was recorded as the MIC. The last vials with no growth were streaked on nutrient agar plates and incubated at 37°C for 24 hours. The lowest concentration that killed 100% of the inoculum bacteria (no growth on plate) was recorded as Minimum bactericidal concentration (MBC).

### **Phytochemical analysis**

Phytochemical analysis of ethanolic extract of *T.chebula* fruit was done for the presence or absence of active secondary metabolites or different constituents such as tannins, alkaloids, flavanoids, terpenoids, steroids, carbohydrates, proteins, saponins etc., The dried extract was reconstituted in DMSO and subjected to standard phytochemical analysis following the procedures of Harborne<sup>22</sup>.

### **Statistical analysis**

All the above assays were conducted in duplicate and repeated thrice for consistency of results and statistical purpose. The mean zone of inhibition and standard deviations were calculated for all treatments.

## **RESULTS AND DISCUSSION**

Urinary tract infection refers to the presence of clinical signs and symptoms arising from the genitourinary tract plus the presence of one or more microorganisms in the urine exceeding threshold value significance (ranges from  $10^2$  to  $10^3$  colony forming units/ml). Single UTI episodes are very common especially in adult women. In addition, recurrent UTIs are also

common, occurring in up to one-third of women after first episode UTIs. A cornerstone of prevention of UTI recurrence has been the use of low-dose once daily or post-coital antimicrobials; however, much interest has surrounded non-antimicrobial based approaches undergoing investigation such as use of medicinal plants, probiotics, vaccines, oligosaccharide inhibitors of bacterial adherence and colonization. Multiple antimicrobial resistances among Gram-negative organisms have been a long term and well-recognized problem with urinary tract infections. Identification of the causative organism and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages of urinary tract infections. Identification of the active component from natural products can lead to the synthesis of more potent analogues that can be readily formulated into more traditional dosage forms<sup>23</sup>. Evidences supporting the beneficial health effects of medicinal plants are indisputable and largely discussed and proved<sup>24</sup>. *T. chebula* is routinely used as traditional medicine by tribals of Tamil Nadu to cure several ailments. The investigation by authors<sup>25,26</sup> validate the traditional use and suggests that *T. chebula* extracts has better efficacy and can be a source for natural antimicrobial agent. Literature on the antimicrobial activity of *T. chebula* fruit and seeds against urinary tract pathogens is limited. In our study, we made an attempt to fill the gap in literature data by using the ethanolic extracts of fruit and seeds of *T. chebula* against urinary tract pathogens. The Enterobacteriaceae were the most frequent pathogens detected, causing 84.3% of the UTIs<sup>27</sup>. Urine samples were collected from the nearest hospital and processed for the isolation of pathogens. Five organisms were identified. Among them, *Escherichia coli* are predominant followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Previous studies had also demonstrated that *Escherichia coli* is the most frequent etiological agent causing community and hospital-acquired UTIs, followed by *Klebsiella*<sup>28,29</sup>.

In the current study the antimicrobial activity of the ethanolic extract of *T.chebula* (Fruit and seed) against UTI pathogens was measured. The tested microbial strains showed different patterns of inhibition. The results were summarized in Table 1 and 2. In this study, both in agar well diffusion and agar disc diffusion technique, the fruit and seed extract showed activity against all the pathogens tested. On comparing the both techniques, the agar-well diffusion technique provided more antimicrobial activity while agar disc diffusion technique showed less



activity against the urinary tract pathogen tested. The results support the earlier investigation by Srinivasakumar and Rajashekhar<sup>30</sup> i.e. crude butanol extract showed total inhibitory activity of 60.3 mm in well cut method and 54.7 mm in the paper disc method. The results clearly indicated significant increment in microbicidal activity when performed using well-cut method.

TABLE 1. Antimicrobial Activity of *Terminalia chebula* Against Urinary Tract Pathogens (Agar Well Diffusion Technique)

Parts used	Concentration of extract used	Microorganisms used (Zone of Inhibition in mm)				
		<i>E.coli</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>
FRUITS	5µl	13±0.4	12±0.5	16±0.2	20±0.1	10±0.3
	10µl	16±0.2	16±0.1	20±0.5	22±0.2	11±0.3
	15µl	20±0.5	18±0.3	22±0.4	24±0.5	13±0.4
	20µl	23±0.3	20±0.2	25±0.3	26±0.2	18±0.5
SEEDS	5µl	08±0.4	10±0.5	12±0.1	20±0.4	08±0.3
	10µl	11±0.6	12±0.3	16±0.3	22±0.2	10±0.4
	15µl	12±0.3	13±0.1	20±0.2	23±0.3	12±0.5
	20µl	14±0.1	15±0.4	21±0.4	25±0.1	13±0.6

Zone of Inhibition = Mean Inhibition Zone ± Standard Deviation in mm

TABLE 2. Antimicrobial Activity of *Terminalia chebula* Against Urinary Tract Pathogens (Agar Disc Diffusion Technique)

Parts used	Concentration of extract used	Microorganisms used (Zone of Inhibition in mm)				
		<i>E.coli</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>C.albicans</i>
FRUITS	5µl	11±0.2	11±0.3	15±0.4	15±0.5	10±0.3
	10µl	13±0.4	13±0.1	17±0.2	20±0.2	12±0.1
	15µl	15±0.3	17±0.3	20±0.5	21±0.3	15±0.4
	20µl	18±0.1	20±0.2	22±0.3	24±0.1	16±0.2
SEEDS	5µl	09±0.3	07±0.4	08±0.4	14±0.2	06±0.5
	10µl	11±0.2	08±0.5	14±0.6	20±0.3	08±0.6
	15µl	12±0.4	10±0.2	15±0.3	22±0.4	09±0.3
	20µl	14±0.1	12±0.5	18±0.1	23±0.2	11±0.5



Positive control Ciprofloxacin	15	17	28	26	-
Fluconazole	-	-	-	-	28
Negative control DMSO	NZ	NZ	NZ	NZ	NZ

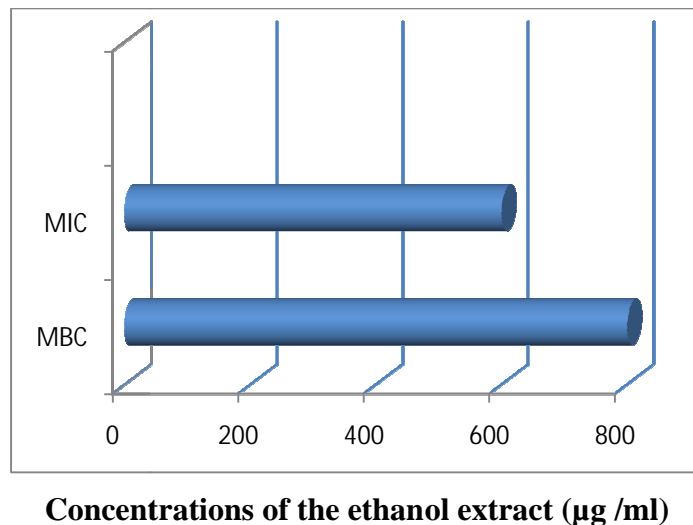
- NZ – No Zone
- Zone of Inhibition = Mean Inhibition Zone  $\pm$  Standard Deviation in mm

In both agar well diffusion and agar disc diffusion technique, we found that ethanolic extracts of fruits showed maximum antibacterial activity against the Gram positive as well as Gram negative urinary tract pathogens than the ethanolic extracts of seeds. The fruit and seed extract of *T. chebula* was strongly inhibited to *S.aureus*, forming large zone of inhibition i.e. 20-26mm in agar well diffusion and 14-24mm in agar disc diffusion technique whereas it showed less activity against *C. albicans* as it formed 8-18mm in agar well diffusion and 6-16mm in agar disc diffusion technique. The present result was in correlation to Kannan *et al.*,<sup>11</sup> who explained the activity of ethanolic extracts of the *T.chebula* fruit showing highest activity towards the bacterial species. The investigation done by Ziaulhaq *et al.*,<sup>31</sup> on *Candida albicans* also supports the present result. Mostafa *et al.*,<sup>32</sup> also reported none of the *T. chebula* extract (Chloroform, ethyl acetate, methanol and ethanol) was able to produce any biocidal effect on yeast; hence the plant material can be better used as an antibacterial agent, rather than an antifungal agent. The study made by Elizabeth<sup>33</sup> on antimicrobial activity of *T. bellerica* dry fruit on certain pathogenic microorganisms also evident that fruit extract was strongly inhibited to *S.aureus*, forming large zone of inhibition i.e. 28 and 30mm respectively. Similar results were obtained with *T. bellerica*, since both plants belong to the same family and fruits taste the same and possessed a few similar phytochemical substances. Gupta *et al.*,<sup>34</sup> reported that *T. pallida* fruit methanolic extract showed maximum activity against gram negative bacteria, while that of *T. bellerica* showed the highest inhibition zones against *Pseudomonas aeruginosa* and *E. coli*<sup>35</sup>. The broad spectrum of antibacterial activity was also reported for *T. arjuna*<sup>36</sup>. It is also important to note that susceptibility of the pathogens varied with the ethanol extract. This indicates the involvement of more than one active principle of biological significance.

Based on the preliminary screening result obtained from agar well diffusion technique and agar disc diffusion technique, the ethanolic extracts of *T.chebula* fruit was found to be most potent antibacterial extract against *Staphylococcus aureus*. The MIC and MBC of the fruit extract

against *S.aureus* was determined by tube dilution method in Muller Hinton broth according to NCCLS<sup>21</sup> and the results are summarized in Figure 2. In this investigation, a different concentration of extracts was used viz. 1000µg, 800µg, 600µg, 400µg, and 200µg/ml respectively. The lowest concentration that exhibited no visible growth was recorded as the MIC. The lowest concentration that killed 100% of the inoculum bacteria (no growth on plate) was recorded as MBC. The MIC of fruit extract against *S.aureus* was recorded as 600µg/ml (no visible turbidity) whereas MBC was recorded as 800µg/ml. (no growth on plate). From this result, we conclude that 800µg/ml was found sufficient to abolish *S.aureus*. This present study showed antibacterial activity at a low concentration, whereas Ahmed *et al.*,<sup>37</sup> reported similar activity at a concentration of 200mg/ml.

Figure 2. MICs and MBCs of ethanol extract of *T. chebula* fruit against *S. aureus*.



Elizabeth<sup>33</sup> reported the MIC values of crude and methanol extracts of *T. bellerica* fruit against *S. aureus* as 300µg and 250 µg, suggesting that *T. bellerica* was most effective against *S. aureus*. Mostafa *et al.*,<sup>32</sup> suggested that the MIC and MBC of the aqueous extract of *T.chebula* against *Salmonella* and *Vibrio cholerae* was 8mg/ml and it was found sufficient to abolish both *Salmonella* and *Vibrio cholerae*.

Similarly, Kannan *et al.*,<sup>11</sup> studied the ethanol extract of *Terminalia chebula* fruit for its antibacterial activity against clinically important standard reference bacterial strains. The MIC was determined as 1 mg/ml for *S. typhi*. This result is correlated with the present study which indicated that the *T. chebula* dry fruit possesses a potential broad spectrum of antimicrobial activity.

The phytochemical analysis of ethanol extract of *Terminalia chebula* fruit revealed the bioactive compounds which are responsible for the invitro antibacterial activity over the pathogens could be Tannins, Quinones, Phenols, Coumarins etc., (Table 3). These results are coinciding with the results revealed by Das *et al.*,<sup>38</sup>. Raju *et al.*,<sup>16</sup> has also reported on presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids in *T.chebula*. Terpenoids from *T. avicennioides* showed antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa*<sup>39</sup>.

TABLE 3. Phytochemical Analysis of Fruit Extract of *Terminalia chebula*

S.No	Phytochemical test	Observation	Inference
1.	Carbohydrates	Red color	++
2.	Tannins test	greenish black color	++
3.	Saponin test	No colour change	-
4.	Flavonoid test	Yellow colour	+
5.	Alkaloid test	No colour change	-
6.	Quinones	Red color	++
7.	Glycosides test	Pink color	+
8.	Cardiac glycosides test	No colour change	-
9.	Terpenoids test	No colour change	-
10.	Triterpenoids	No colour change	-
11.	Phenols	Blue colour	++
12.	Coumarins	Yellow color	++
13.	Proteins	No colour change	-
14.	Steroids and Phytosteroids	No colour change	-
15.	Phlobatannins	No colour change	-
16.	Anthraquinones	No colour change	-

+ Present; ++ Strongly Present; – Absent

Sato *et al.*,<sup>40</sup> 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 antimicrobial studies revealed that the ethanolic extract of *T.chebula* fruit exhibited anti-*Staphylococcus*, anti-*Pseudomonas* and anti-*E.coli* profile. The results indicated the ethanolic extract could be used for the treatment against Urinary tract pathogens.

The investigation done by various authors<sup>41, 42</sup> highlight the fact that the organic solvent extracts exhibit greater antibacterial activity because the active principles were either polar or non-polar and were extracted only through successive organic solvents. Two possibilities that may account

for the higher antibacterial activity of alcoholic extracts are the nature of biological active components (alkaloids, flavonoids, essential oil, terpenoids, 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<sup>35</sup>. The inhibitory effect of fruit extracts of *T. bellerica* can be attributed to the chemical substances (gallic acid and ethyle gallate) that were present in the fruits. Tannins may also be present as in case of *T. chebula* fruits which possessed tannin-B<sup>43</sup>.

Among bacterial pathogens, gram positive bacterial strains were found to be more susceptible than gram negative bacterial strains. This may be attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas, gram negative cell wall is multilayered structure bounded by an outer cell membrane. 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.

Thus, results from the previous and present studies have established that *T. chebula* is a potential candidate.

## CONCLUSION

From this research report, I conclude that ethanolic extracts of *T.chebula* fruits are active against urinary tract pathogens. Such a potential of this medicinal plant, therefore demands further research to unfold its therapeutic values. Further purification and fractionation of the compound is necessary to evaluate the antimicrobial activity of the pure compound. The toxicological studies of the plant and in vivo trials should be carried out. So that it can be used as a potential source for the development of a phytomedicine to act against urinary tract pathogens. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of non toxic plant products having traditional medicinal use, development of modern drugs from *T. chebula* should be emphasized for the control of urinary tract infections. Besides, the same may also be used for self medication in domestic settings.

## ACKNOWLEDGEMENTS

The authors are thankful to the Management of MSCAS for providing laboratory facilities.

## REFERENCES

1. Heuer O.E., Hammerum A.M., Collignon P., Wegener H.C., "Human health hazard from antimicrobial resistant enterococci in animals and food", *Clinical Infectious Diseases*, 2006;43:911-916.
2. Westh H., Zinn C.S., Rosdahl V.T., "An International multi cancer study of antimicrobial consumption and resistance in *S. aureus* isolates from 15 hospitals in 14 countries", *Microbe Drug Resistance*, 2004; 10:169-176.
3. Scazzocchio F., Cometa M.F., Tomassini L., and Palmery M., "Antibacterial activity of Hydrastis Canadensis extract and its major isolated alkaloids", *Planta. Med.*, 2001; 67:561-563.
4. Prusti A., Mishra S.R., Sahoo S., Mishra S.K., "Antibacterial Activity of Some Indian Medicinal Plants", *Ethnobotanical Leaflets*, 2008; 12: 227-230.
5. Murugesan S., Pannerselvam A., Tangavelu A. C., "Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burm.", *F. J. Appl. Pharm. Sci.*, 2011; 1:42-45.
6. Jabeen R., Ashraf M., Ahmad I., "Evaluating the effects of cold water diffusates against *Xanthomonas oryzae* Pv. *Oryzae* causing bacterial leaf blight in rice", *Archives of Phytopathology and Plant Protection*, 2007;40:1-9.
7. Banso A., "Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*", *J. Medicinal Plants Research*, 2009; 3:82-85.
8. Ahameethunisa A. R., Hopper W., "Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria", *BMC Complementary and Alternative Medicine*, 2010;10:1-6.
9. Bag A., Chattopadhyay R.R., "Evaluation of antibacterial properties of Chebulic myrobalan (fruit of *Terminalia chebula*) extracts against methicillin resistant *Staphylococcus aureus* and trimethoprim-sulphamethoxazole resistant uropathogenic *Escherichia coli*", *Afr. J. Plant Sci.*, 2009;Vol.3(2):025-029.
10. Gaidhani S.N., Lavekar G.S., Juvekar A.S., Sen S., Singh A., Kumari S., "In-vitro anticancer activity of standard extracts used in ayurveda", *Pharmacognosy Magazine*, 2009; 5:425-429.
11. Kannan P., Ramadevi S.R., Hopper W., "Antibacterial activity of *Terminalia chebula* fruit extract", *African Journal of Microbiology Research*, 2009; 3:180-184.

12. Rao N.K., Nammi S., “Antidiabetic and renoprotective effects of the chloroform extract of *Terminalia chebula* Retz. seeds in streptozotocin-induced diabetic rats”, BMC Complement. Altern. Med., 2006; 7: 17.
13. Vidya S.,” Hepato-Protective Activity of *Terminalia Chebula* Leaves in Paracetamol Induced Hepato-Toxicity in Rats: 4”, International Journal of Advance in Pharmaceutical Research, 2011; 2: 127-132.
14. Kim H.G., Cho J.H., Jeong E.Y., Lim J.H., Lee S.H., “Growth inhibitory activity of active component of *Terminalia chebula* fruits against intestinal bacteria, J. Food Prot., 2006;Vol.69(9):2205-2209.
15. Chattopadhyay R.R., Bhattacharyya S.K., Medda C., Chanda S., Datta S., Pal N.K., “Antibacterial activity of black myrobalan (fruit of *Terminalia chebula* Retz.) against uropathogen *Escherichia coli*, Phcog Rev., 2007;11: 212-215.
16. Raju D., Ilango K., Chitra V., Ashish K., “Evaluation of Anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats”, J. Pharm. Sci. and Res., 2009;1: 101-107.
17. Cappucino C James., Natalie Sherman., Microbiology Laboratory Manual, 2007; 7<sup>th</sup> edition.
18. Cheesebrough M., Medical Laboratory Manual for Tropical Countries, Linacre House, Jordan Hill, Oxford, 2000; 260.
19. Saeed S., Tariq P., “Antibacterial activities of *Embllica officinalis* and *Coriandrum sativum* against gram negative urinary pathogens”, Pak. J. Pharm. Sci., 2007; Vol.20 (1): 32-35.
20. Bauer *et al.*, 1966
21. National committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests NCCLS document M2-A7, Approved Standard, 7th edition: Wayne, PA: NCCLS, 2000.
22. Harborne J.B., Phytochemical Methods, Chapman and Hall Publications, London, 1998; 7-8.
23. Ayoola G.A., Coker H.A.B., Adesegun S.A., Adepoju-Bello A.A., Obaweya I.K., Ezennia E.C., Atangbayila T.O., “Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria”, Tropical Journal of Pharmaceutical Research, 2008; 7 (3): 1019-1024.

24. Lee S.S., Zhang W., Li Y., “The antimicrobial potential of 14 natural herbal dentifrices: Results of an in vitro diffusion method study”, J. Am. Dent. Assoc., 2004; 135: 1133- 41.
25. Meng J.C., Zhu Q.X., Tan R.X., “New antimicrobial mono- and sesquiterpenes from *Soroseris hookeriana* subsp. *erysimoides*”, Planta. Medica., 2000; 66: 541–544.
26. Mimica-Dukic N., Kujundzic S., Sokovic M., Couladis M., “Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill obtained by different distillation conditions”, Phytother. Res., 2003; Vol. 17(4):368-71.
27. Gales C.A., Jones R.N., Gordon K.A., Sader H.S., Wilke W.W., Beach M.L., Pfaller M.A., Doern G.V., SENTRY Study Group (Latin America), “Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY Antimicrobial Surveillance Program (1998)., J. Antimicrob. Chemotherapy, 2000; 45: 295-303.
28. Blot S., Bacquer D.D., Hoste E., Depuydt P., Vandewoude K., Waele J.D., Benoit D., Schuijmer J., Colardyn F., Vogelaers D.,” Influence of matching for exposure time on estimates of attributable mortality caused by nosocomial bacteremia in critically ill patients”, Infect. Control Hosp. Epidemiol., 2005; Vol. 26(4): 352-356.
29. Kay C.D., Holub B.J.,”The effect of wild blue berry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects”, Br. J. Nutr., 2005;88: 389-397.
30. Srinivasakumar K.P., Rajashekhar M.,” In vitro studies on bactericidal activity and sensitivity pattern of isolated marine microalgae against selective human bacterial pathogens”, Indian Journal of Science and Technology, 2009; Vol.2(8):16-23.
31. Ziaulhaq M., Ahmad M., Mehjabeen, Jehan N., Ahmed S., Qayum M., Marwat I.K., “Antimicrobial screening of selected flora of Pakistan”, Arch. Biol. Sci., Belgrade, 2011; Vol.63 (3): 691-695
32. Mostafa M.G., Mahdia Rahman M., Karim M.M., “Antimicrobial Activity of *Terminalia chebula*”, Indian Journal of Medical Aromatic Plants, 2011; Vol. 1(2): 175-179.
33. Elizabeth K.M., “Antimicrobial activity of *Terminalia bellerica*”, Indian Journal of Clinical Biochemistry, 2005; Vol. 20 (2): 150-153.
34. Gupta M., Mazumder U.K., Manikandan L., Bhattacharya S., Haldar P.K., Roy S., “Antibacterial activity of *Terminalia pallida*”, Fitoterapia, 2002; 73:165-167.



35. Ghosh A., Das B.K., Roy A., Mandal B., Chanda G., “Antibacterial activity of some medicinal plant extracts”, J. Nat. Med., 2008; 62:259-262.
36. Singh D.V., Gupta M.M., Santha K.T.R., Saikia D., Khanuja S.P.S., “Indian ocean dipole mode and tropical cyclone frequency”, Current Science, 2008; Vol. 94(1):10.
37. Ahmad I., Mehmood Z., Mohammad F.,” Screening of some Indian medicinal plants for their antimicrobial properties”, J. Ethnopharmacology, 1998; Vol.62 (2): 183-193.
38. Das K., Tiwari R.K.S., Shrivastava D.K., “Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends”, Journal of Medicinal Plant Research, 2010; Vol. 4(2):104-111.
39. Mann A., Amupitan J.O., Oyewale A.O., Okugun J.I., Ibrahim K., “Antibacterial activity of terpenoidal fractions from *Anogeissus leiocarpus* and *Terminalia avicennioides* against community acquired infections”, Afr. J. Pharm. Pharmacol., 2009; Vol. 3(1):22-25.
40. Sato Y.H., Odetani K., Singyouchi T., Ohtsubo M., Kihara Shibata H., Higuti T.,”Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* Retz. against methicillin-resistant *Staphylococcus aureus*”, Biol. Pharm. Bull., 1997; 20:401-404.
41. Jayalakshmi B., Raveesha K.A., Amruthesh K. N., “Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria”, Journal of Applied Pharmaceutical Science, 2011; Vol.1 (5): 124-128.
42. Mohanasundari C., Natarajan D., Srinivasan K., Umamaheswari S.A., Ramachandran A., “Antibacterial properties of *Passiflora foetida* L., a common exotic medicinal plant”, African J. Biotechnol., 2007; 6: 2650- 2653.
43. Rastogi P., Mehrotra B.N., “Compendium of Indian Medicinal plants, Drug Research perspective, CDRI Lucknow and NISCOM, New Delhi, 1999;2:1-859.