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Antimicrobial Activity of *Terminalia chebula* Extract against Urinary Pathogens

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Abstract

Background: *Terminalia chebula* (Lalob) is a medicinal plant widely distributed throughout India, Burma and Srilanka. The dried, ripe fruit of *Terminalia chebula* (*T. chebula*), also known as black myrobalan has widely been used in the treatment of asthma, sore throat, vomiting, hiccough, bleeding piles, diarrhoea, gout, heart diseases, and bladder infections. *T. chebula* is a popular medicinal plant and has a broad spectrum medicinal antibacterial action.
Objective: To assess the antibacterial activity of *Terminalia chebula* extract against urinary pathogens.
Materials and methods: *T. chebula* fruits were collected locally and processed at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (Khartoum). Using the cup plate method the antibacterial activity of *T. chebula* extract was tested with different concentrations against the organisms: *Escherichia coli, Klebsiella pneumoniae. Pseudomonas aeruginosa,* and *Proteus* spp. The antibiotic discs used were ciprofloxacin, imipenem, and gentamicin.
Results: The extract of *Terminalia chebula* showed a clear antibacterial activity against the urinary pathogens. This activity was enhanced with increasing concentrations of *Terminalia chebula* .100 % concentration of *Terminalia chebula* extract was found to give the best antibacterial activity against *Escherichia coli, Klebsiella pneumoniae. Pseudomonas aeruginosa,* and *Proteus* spp.
Conclusion: The100% concentration of *Terminalia chebula* extract had the best antibacterial activity against the urinary pathogens investigated in this study.

Key words: *Terminalia chebula* extract, Antibacterial activity, Urinary pathogens.

Introduction

The discovery of antibiotics 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. We are now facing a threat of pathogenic bacteria resistant to most or all available antibiotics. It was warned by the World

Health Organization (WHO) that such multiple antibiotic-resistant pathogens would 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 enormous demand has triggered worldwide efforts in developing novel antibacterial alternatives, particularly the screening of several medicinal plants for their potential antimicrobial activity. Many Bangladeshi plants had been used from time immemorial to treat various diseases and infections in traditional medicine[1].

Herbal medicines are in great demand in developed as well as in developing countries for primary health care because of their wide biological and medicinal activities, higher safety margin, and cheap costs. *T. chebula* (Lalob) has been reported to have antioxidant and free radical scavenging activities . It is active against cancer cells and *Helicobacter pylori*. It is also useful as an anti carrier agent and used in dermal wound healing and improving the gastrointestinal motility. It is also used in anaphylactic shock, in diabetes mellitus, and found to be a strong antimicrobial agent against the uropathogen *Escherichia coli*. Gallic acid and its ethyl ester isolated from ethanolic extract of *T. chebula* showed an antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. It has also a growth inhibitory action against *Salmonella typhi* and other intestinal bacteria[2].

To the best of our knowledge, there was no published reports in Sudan regarding the antimicrobial activity of aqueous extract of lalob fruits. Hence the object of this study was to assess the antibacterial activity of *Terminalia chebula* aqueous extract against urinary pathogens.

Materials and methods

T. chebula fruits were collected locally and were processed at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (Khartoum). The fruits were air-dried under the shadow with good ventilation and then finely grounded. The extract of *T. chebula* was prepared by mixing 20 g of *Terminalia chebula* fruit powder with 100 ml ethanol and left undisturbed overnight. Then the mixture was filtered twice using No. (1) Whatsman filter paper. The clear filtrate was condensed using a rotary vacuum evaporator at 250°C for about 15 min.

The bacterial pathogens used in this study were isolated from urine specimens. Urine specimens were cultivated on blood agar and Mac Conkey agar and incubated overnight at 37°C for 24 hr. Identification of bacterial species was performed using standard bacteriological conventional techniques. Bacterial identification was carried out by conventional biochemical methods according to the standard microbiological techniques. The series of biochemical tests used for identification were: colonial morphology, Gram's stain, catalase test, mannitol fermentation test, oxidase test, citrate test, urease test, and indole test. Organisms isolated were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus* spp. All tests were conducted according to the Bergey's Manual of Determinative Bacteriology[3 -11].

The susceptibility patterns of the bacterial species isolated were determined twice: against the routine commercial antibiotics and against *Terminalia chebula* extract.

(1) Antibiotics susceptibility patterns: This was determined by Kirby Bour disc diffusion technique. Mueller Hinton agar (Oxoid) was used to determine the sensitivity pattern of bacteria. This medium was prepared according to the manufacturer's instructions. It was sterilized by autoclaving at 121ºC for 15 minutes and poured in sterile Petri plates. *In vitro* susceptibility of the bacterial isolates to various antimicrobial agents. A loop-full colony from each bacterial isolate was inoculated on nutrient agar and incubated at 37°C for 24 hours. The bacterial suspension of each isolate was prepared with normal saline. The suspension turbidity was adjusted and compared with the Mc Farland standard to give a suspension containing 1.5 $x10^8$ c fu/ml. A cotton swab was dipped into each bacterial suspension and streaked on Mueller Hinton agar plate, left for 10 minutes at room temperature to dry. The antibiotic discs were placed on the surface of the medium with sterile forceps and pressed gently to ensure good contact with the surface of the medium. The plates were incubated at 37°C within 15 minutes after applying the discs. After 18 hours incubation, each plate was examined to determine the zones of inhibition of the antibiotics. The antibiotic discs used were ciprofloxacin (5 µg), imipenem (10 µg), and gentamicin (10 µg).
(2) *Terminalia chebula* susceptibility patterns: The antibacterial activity of *Terminalia chebula* was tested by the cup-plate agar diffusion method with some minor modifications. The bacterial suspension was thoroughly mixed with 1ml sterile normal saline, compared with Mc Farland standard, and streaked on Muller Hinton agar plate. Wells (measuring 8 mm in diameter) were cut out of the Mueller-Hinton agar under aseptic conditions using sterile blue tubes. Each well was filled with 20 µl of the *Terminalia chebula* extract at the concentrations: 100%, 50%, 25%, and 10%. The plates were refrigerated for 2 hours to allow proper diffusion before incubation in an upright position at 37°C for 24 hours. Then the plates were removed from the incubator and under good illumination the inhibition zones around the wells were measured.

Results

Regarding the antibiotics susceptibility pattern, imipenem was found the most effective antibiotic against all bacterial species isolated. Also *E.coli* was the most sensitive organism to all antibiotics used in the study (Table 1).

Table (1): Antibiotics susceptibility patterns of the bacterial species isolated

Antibiotics	Inhibition zones in mm		
	E. coli	*K. pneumoniae*	*Proteus* spp
Ciprofloxacin	20	10	13
Imipenem	33	25	30
Gentamicin	23	18	24

On the other hand, the antimicrobial activity of different concentrations of *Terminalia chebula* extract was performed by the well agar diffusion assay method. The isolates investigated were

Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and *Proteus* spp. The different concentrations of *Terminalia chebula* extract (100%, 50%, 25%, and 10%.) showed variable ranges (mean/standard deviation) of antibacterial activity. *Escherichia coli* was the most sensitive strain to the extract of *T. chebula*, with an inhibition zone of 9 mm at an extract concentration of 100%. Also *K. pneumoniae* and *Proteus* spp. had exhibited wide sensitivity zones (17 mm and 15 mm respectively) to 100% extract concentration of *T. chebula* (Table 2).

Table (2): *Terminalia chebula* susceptibility patterns of the bacterial species isolated

T. chebula concentration 4	Inhibition zones in mm		
	E. coli	*K. pneumoniae*	*Proteus* spp
100%	19	17	15
50%	15	15	10
25%	13	10	7
10%	11	5	-

Discussion

Herbs have a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have antimicrobial properties *in vitro*. Tannin is a general descriptive name for a group of polymeric phenolic substances. Many physiological activities such as stimulation of host mediated tumor activity and a wide range of anti infective actions have been assigned to tannins. Their mode of antimicrobial action may be related to their ability to inactivate microbial enzymes and transport proteins.

In this study, imipenem was found the most effective antibiotic against all bacterial species isolated, and *E.coli* was the most sensitive organism to all antibiotics tested. Also, the different concentrations of *Terminalia chebula* extract showed variable ranges of antibacterial activity. *Escherichia coli* was the most sensitive strain to the extract of *T. chebula* at 100% concentration; as well as *K. pneumoniae* and *Proteus* spp. which had exhibited wide sensitivity zones (17 mm and 15 mm respectively) to the same 100% extract concentration of *T. chebula.*

Malekzadeh and his colleagues[3] studied the water extract of *T. chebula* and they reported significant antibacterial activity with a minimum inhibitory concentration of 125 mg/l.

Also Sangeetha[4] investigated *Terminalia chebula* by both agar well diffusion and agar disc diffusion techniques against the urinary tract pathogens *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. In both techniques, they found that *T. chebula* extract of fruits showed maximum antibacterial activity against the Gram positive as well as Gram negative urinary tract pathogens.

Furthermore, Datta and his co-authors[5] were able to evaluate the antibacterial activity of *T. chebula* using agar well diffusion assay method against some common gram positive and gram negative bacteria. They reported that *Terminalia chebula* was found to be effective against *Pseudomonas aeruginosa* and *Escherichia coli.*

Conclusion: The100% concentration of *Terminalia chebula* extract had the best antibacterial activity against the urinary pathogens investigated in this study

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