ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(4): 485-488

ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of Hydroalcoholic Extract of *Terminalia chebula* Retz. on Different Gram-positive and Gram-negative Bacteria

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Received 28 Oct 2010; Revised 21 Nov 2010; Accepted 30 Nov 2010

ABSTRACT

The present study describes the anti-bacterial activity of hydro-alcoholic extract of *Terminalia chebula Retz* fruit against microorganism like: *Bacillus substils, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Staphylococcus flexineria* and *Pseudomonas aeruginosa*. For this purpose hydro-alcoholic extract of fruit were prepared and tested by "Disc Diffusion Method". As a result of this study it was found that the extract of fruit generally revealed anti-bacterial activity against both gram-positive bacteria (*B. subtils, S. aureous* and *S.epidermis*) and gram-negative bacteria (*E. coli, S. flexineria and S. auriginosa*).

Key words: Anti-bacterial activity, *Terminila chebula*, disc diffusion method.

INTRODUCTION

Herbal medicines are in great demand in the developed as will as in developing countries for primary heath care because of their wide biological and medicinal activities, higher safety margin and lower costs. *Terminila chebula Retz* (*combretaeae*) is medicinal plant widely distributed through out India, Burma and Srilanka. The dried ripe fruit *T. chebula* also know as black myrobalan has widely been used in the treatment of asthma, sore throat, vomiting, hiccough, bleeding, piles, diarrhoea, gout, heart and bladder diseases.[1] Black myrobalan has reported to have antioxidant and free redical scavenging activities. [2] It is active against cancer cells [3] and *Helicobactor pyroli*. [4] It is also useful as an anticarries agent. [5] It used in dermal wound healing [6] and improving gastrointestinal motility. [7]

It is reported to use in anaphylactic shock[8] and in diabetes mellitus.[9] One group of researchers found that both aqueous and ethanolic extract of *T. chebula* have strong anti-bacterial activity against the uropathogen *Escherichia coli*.[10] Gallic acid and its ethyl ester isolated from ethanolic extract of *T.chebula* showed anti-bacterial activity against methicillin resistance *Staphylococcus aureus*. [11] It has also growth inhibitory action against *Salmonella typhi* and intestinal bacteria. [11] To the best of our knowledge, there have been no published reports concerning the anti-bacterial activity of hydro-alcoholic extract of *T.chebula* fruits against *B. subtilus, S. aureous, S.epidermis, E.coli, S. flexineria* and *P.auriginosa*. We have, therefore focused on its anti-bacterial activity in our study. This was achieved with the disc diffusion method.

MATERIAL AND METHODS

Plant materials

T.chebula fruits were purchased from local market and were Authetified by the department of Botany, Bharathidasan University, Thiruchirappalli, India. The herberium Sheet was submitted in department of Botany at Bharathidasan University, Thiruchirappalli, India.

Preparation of extract

The *T. chebula* fruits were dried and coarsely powdered. The dried coarsely powder of fruits were extracted with water and ethanol mixture (50:50) for 20 Hrs. in a iodine flask. The solvents were removed in rotary evaporator and the crude extracts were dried at room temperature in a steady air current. The dried extracts were then

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stored in airtight jars at 4°C for anti-bacterial analysis.

Phytochemical studies-

The preliminary phytochemical screening was done for the presence of alkaloid, tannins, flavonoid, terpenes and saponine.[12]

Formulation of extract

For anti-bacterial activity, on the day of experimentation, the different amount of extract was suspended in distilled water to get different concentration of extract.

Microorganism

The microorganism used in this study was *B.subtilus(), S.aureous*(ATCC25923), *S.epidermis(), E.coli*(ATCC25922), *S.flexineria(), P.auriginosa*(ATCC10145). The microorganisms were procured form the National Chemical Laboratory (NCL) Pune, India. Microorganisms were maintained at 4°C on nutrient agar slants.

Anti-bacteria activity

The anti-bacterial activity of the hydro-alcoholic extract was carried by disc diffusion method.[12] A suspension of tested microorganisms was spread on Muller-Histon Agar (MHA) (Oxoid) medium. The filter paper discs (5mm in diameter) were individually impregnated with different concentration of extract and then placed into the agar plates which had previously been inoculated with the tested microorganisms. The plates were subsequently incubated at 37° C for 24 Hrs. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. All the tests were performed in triplicate. Tobramycin, cephalexin, erythromycin and ampicillin served as positive control.[12]

RESULT AND DISCUSSION

In-vitro preliminary screening of the anti-bacterial activity of the plant extracts from *T.chebula* was studied against some bacteria using the filter paper disc diffusion method. The anti-bacterial affect of plant extract against the different strains are illustrated in Table and Figure. The extract of *T.chebula* at the concentration of 100% has anti-bacterial activity on the tested microorganism form high to low respectively. *P.auriginosa* (19mm), *E.coli* (16mm), *S. epidermis* (15mm), *S. aureus*(15mm), *S. flexineria*(14mm), and *B.subtilus* (14mm) showed in **(Table -1)**.

Table-1 shows that the extracts of *T. chebula* have activity on *B.subtilus, S.aureaus, S. epidermis, E. coli, S. flexineria , P. auriginosa* (14mm, 15mm, 15mm, 16mm, 14mm, and19mm inhibition zone respectively).

The data indicated that gram negative *P. auriginosa* was the most sensitive strain of those tested with the extract of *T. chebula*, with strongest inhibition zone of 19mm. The extract concentration of 100% also exhibit high anti-bacterial activity against *E.coli.*, with modest activity against *S.epidermis, S. aureaus, B.sustilus, S. flexineria.* The 75% concentration of the extract of *T.chebula* also show strongest inhibition zone against different strains of microorganisms. The data indicates that anti-bacterial activity of extract (at 75% concentration) with strongest inhibition zone of 14mm for the strain of *P.auroginosa, E.coli,* and *S.epidermis*. However, the inhibition zone for the species of *B.subtilus, S. aureaus* and *S.flexinaria* was found 13mm, 12mm and 11mm respectively.

The Table-1 shows that different concentration (50%, 75% and 100%) of plant extract were having good anti-bacterial activity against *B.subtileaus, S.aureaus, S.epedermis, E.coli, S.flexineria* and *P.auriginosa*.

The minimum inhibitory concentration (MIC) of the *T. chebula* fruit extract was meausered which is depicted in the Table-1. It was observed that *S. aureaus* and *P. auriginosa* have shown MIC value at 1% concentration of plant extract. Other bacteria have shown very small zone at 1% concentration of the extract.

On comparing the inhibition zone of the extract to that of standard antibiotics (tobramycin, cephalexin, erythromycin and amoxicillin) extract showed better activity than tobramycin and cephalexin. However, extract is not potent than erythromycin and amoxicillin in these condition.

In the end of study we have found the extract *T.chebula* revealed anti-bacterial activities against microorganisms.

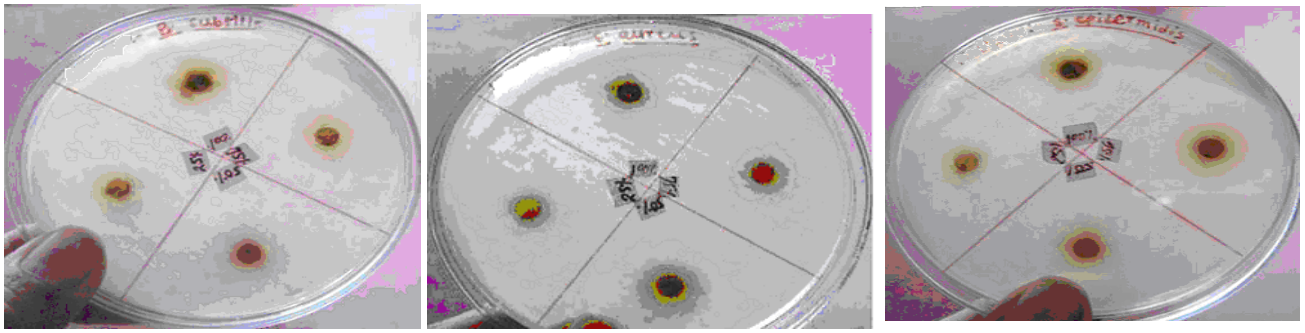
Table 1 : Anti-bacterial activity of *T. chebula* hydroalcoholic extract of different Microorganism.

Sample concentration in Microgram	Microorganism (inhibition zone in mm)					
		Gram-positive			Gram-negative	
	B.subtilus	*S. aureus*	*S. epiderms*	*E.coli*	*S.flexineria*	*S.auriginosa*
100	14	15	15	16	14	19
75	13	12	14	14	11	14
50	12	11	12	13	10	13
25	11	9	11	12	10	12
20	10	9	10	11	8	11
15	10	5	10	9	5	10
10	6	4	10	7	4	6
5	5	3	5	6	3	5
1	-	1	-	-	-	-

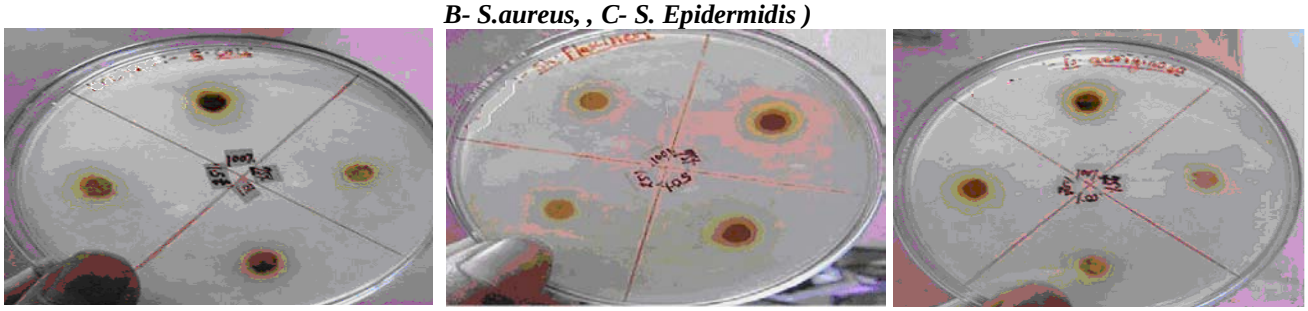
Table 2: Anti-bacterial activity of antibiotics or different Microorganism.

Antibiotics	Microorganism (inhibition zone in mm)					
		Gram-positive			Gram-negative	
	B.subtilus	*S. aureus*	*S. epiderms*	*E.coli*	*S.flexineria*	*S.auriginosa*
Cephalexin	15	20	16	-	-	-
Tobramycin	-	-	-	-	-	-
Ampicillin	7	10	7	-	-	-
Erythromycin	7	8	6	-	-	-

-: Absence of inhibition



Photograph showing the zone of inhibition of *T. chebula* extract against various Microorganisms, *A- B. subtilis, B- S.aureus, , C- S. Epidermidis)*



Photographs showing the zone of inhibition of *T. chebula* extract against various *Microorganisms A- E. coli, B- shigella floxineri, C-P. aeriginosa.*

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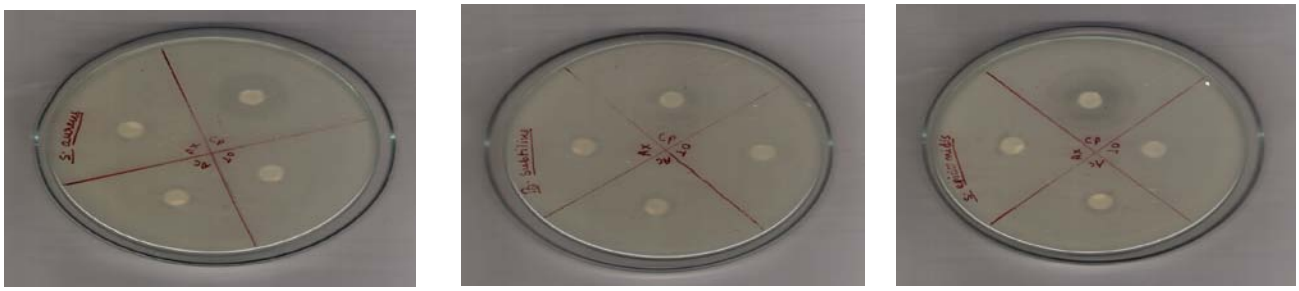

Photographs showing the Antibacterial activity of Standard antibiotic against different micro organism

CONCLUSION

In conclusion it may be said that hydro-alcoholic extract of *Terminalia chebula* Retz. Fruits can be used for protection against gram positive and gram-negative bacteria. However, further studies on the extract are needed to pinpoint the finding. This report may serve as a footstep on this aspect. This anti microbial activity may be due to presence of alkaloid, flavonoid and terpenes.

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