Therapeutical Effect of Extracts of Terminalia *chebula* In Inhibiting Human Pathogens and Free Radicals

Dolly Singh, Deepti Singh, Soon Mo Choi, Sun Mi Zo, Saet Byul Ki, and Sung Soo Han

***Abstract*— In recent years, multiple drug resistance has been developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Conventional antibiotics are strong medicines, which if not used in precise way may cause harmful effects. The major thrust is to establish alternative antimicrobial agent in order to treat microbial infections with less or no toxic effect to body and less negligible side effects. The herbal medicines have shown potential to overcome the limitation associated with conventional drugs. However, the appropriate choice of source for herbal medicine is also very important. *Terminalia chebula* possesses potential pharmaceutical activities and used in several Ayurvedic formulations. The study of solvent-free organic (ethylacetate, acetone, methanol of increasing polarity) and aqueous extracts of the fruits of *T. chebula*, showed the potential to reduce growth of microorganisms, minimizing the risk of infection, while optimizing the conditions to encourage healing. In our study we found, methanol extract as a potential bactericidal and potent antioxidant while aqueous extract showed the least potential as an antimicrobial agent, though a moderate antioxidant. The finding may provide scientific rationale for the use of crude extract of the plant as a new drug compound as potential antioxidant and antimicrobial agent.**

***Index Terms*—Antioxidant, Ayurvedic, Bactericidal, Terminalia chebula.**

I. Introduction

Many infectious diseases like meningitis, bacteremia, pneumonia, scalded skin syndrome etc are caused by most commonly yet pathogenic bacteria belonging to *Streptococcus* and *Staphylococcus* genera [1]-[5]. The resistance of these microorganisms due to excessive use of antibiotics over the years has led to the development of more aggressive and pathogenic strains of them. There is an urgent need for development of effective therapeutics against these resistant strains. Traditional medicinal plants have many therapeutic properties, are accessible by poor community of the world and are economically effective means of treatments for many deadly diseases [6]. Several plant extracts has been tested for their anti-microbial properties world-over [6]-[8].

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Sung Soo Han is with the Dept of Nano, Medical, and Polymer Materials Yeungnam University 214-1 Daedong, Gyeongsan 712749, Korea . (Tel.: +82-53-810-2773, fax: +82-53-810-4685 e-mail:sshan@yu.ac.kr)

Dolly Singh, Deepti Singh, Soon Mo Choi, Sun Mi Zo, Saet Byul Ki are with Dept of Nano, Medical, and Polymer Materials Yeungnam University 214-1 Daedong, Gyeongsan 712749, Korea (e-mail: dollys@yu.ac.kr, deeptis@yu.ac.kr, debate311@nate.com, koy41@nate.com, 87ksb@hanmail.net).

Terminalia chebula is used as a mild laxative and as an astringent against wounds and abscesses. Practictioners of folk medicine in India and Southeast Asia use the fruit for homeostatic, laxative and as cardiotonic. It is used as a remedy against a sore throat and cough, against diarrhoea connected with a prolapsed rectum in China and against ulcers and dysentery in Tibet. The antibacterial activity of various extracts of *Chebula myrobalan* powder was tested against *Streptococcus* species, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

II. Materials

T. chebula powder was purchased from local market of Mumbai, India. Pathogens of MTCC grade were obtained from IMTECH (Chandigarh, India). Gallic acid and Glutaraldehyde was purchased from s.d. fine-chemicals limited (Mumbai, India). Gentamicin and 2,4,6-tripyidyl-s-triazine (TPTZ) were procured from Hi-Media(Mumbai,India), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) reagent, 4'-6-diamidino-2-phenylindole (DAPI), propidium iodide (PI) and nystatin was purchased from Sigma (St. Louis, USA). Fetal bovine serum (FBS) and streptomycin-penicillin antibiotic solutions were bought from HyClone (Utah, USA). All other chemicals used were of analytical grade. SIRC (rabbit corneal epithelial cells) cell line was obtained from NCL, Pune, India

III. Methods

Preparation of *T. chebula* extracts: 25 g of powder of fruits of *T.chebula* was taken in a thimble. Extracts were prepared in the series of 500ml of different solvents based on increasing polarity (Ethyl acetate, Acetone, Methanol, and Water) using Soxhlet extraction method. The soxhletting cycle was completed after the solvents turned colorless in the tube indicating the complete extraction of the phytosignatures. The solvents were evaporated to dryness using rotary evaporator and the extract obtained was lyophilized at –50 ℃. The lyophilized solvent-free extracted powder was stored in airtight bottles at 4 ℃ till further experimentation. [9].

A. Determination of Antimicrobial Activity of T.chebula Extracts

Antibacterial activities of all the solvent free extracts of *T.chebula* (ethyl acetate S1, acetone S2, methanol S3 and aqueous-S4) were determined by agar well diffusion method. The extracts were dissolved in DMSO (dimethylsulphoxide).

The microbial lyophilized cultures were revived at 37 ℃ for 18h in a broth medium and the culture was adjusted to 5 x 10^5 cfu/ml in accordance with the McFarland Turbidity standards [10]. The 20 µl of the culture was spread on Mueller Hinton agar plates and wells of 9mm diameter were punched into the agar plates. 100 µl of the solvent-free extracts of concentration 0.5mg/100 µl and 1mg/100 µl were used for determination of ZOI (Zone of Inhibition). After holding the plates at room temperature for 2 hours to allow diffusion of the extract into the media, the plates were incubated at 37 ℃ for 24h. DMSO and Gentamicin was used as a negative control and reference antibiotic (positive control) respectively. The test was performed in triplicates and the final results were presented as the mean zone of inhibition [11]. Inhibition Zone was observed after 24h and Zone of inhibition was recorded for all the extracts and gentamicin. Inhibition Zone Diameter (IZD) was measured to the nearest millimeter (mm) by reducing the IZD value with Diameter of the well bored.

Total Zone of Inhibition = Inhibition Zone Diameter- Diameter of the well.

B. Determination of Minimal Inhibitory Concentration (MIC)

The Minimal Inhibitory Concentration (MICs) of the extracts of the plant was determined against the tested bacteria by macro broth dilution assay method [12]. Two-fold serial dilutions of all the extracts (based on their IZD results: 0.5mg/100 µl) were prepared in 24well plates with MHA as diluents. Each dilution was seeded with 20 µl of test microorganisms to the standard concentration (5 x 10^5 cfu/ml). Two-fold serial dilution of Gentamicin was used as experimental positive control. The plates were incubated at 37 ℃ for 24h. The least concentration of the extract or standard drug showing no visible growth was taken as the MIC. After 24h of incubation period, mean MIC values were calculated. The test was performed in triplicates for each microorganism used and the final results were expressed as the arithmetic average of triplicate experiments. 20 µl test media from each MIC broth tube was spread over the MHA plates. Plates were incubated at 37 ℃ for 24h. The test MIC concentration showing no bacterial growth on agar plates was considered as Minimum Bactericidal Concentration (MBC) of the extract [11].

C. Determination of Antioxidant Activity of T. chebula Extracts

The ferric-reducing antioxidant power (FRAP) assay measures the antioxidant potentials of “antioxidants” to reduce the Fe3+/ 2,4,6-tripyidyl-s-triazine (TPTZ) complex present in stoichiometric excess to the blue colored Fe2+-TPTZ form. (22). The stock solution of various extracts of concentration 0.3mgml^{-1} was prepared. 10 µl-100 µl (6 X 10-4 g/l - 60 X 10-4 g/l) of extract was mixed with 1.5 ml of FRAP reagent and the volume was adjusted to 5ml with distilled water. The test tubes containing the test solutions were incubated at 37 ℃ for 15min. The absorbance was recorded at 593nm [13].

IV. Results

A. Determination of antimicrobial activity of T.chebula extracts

Among all the extracts of *T. chebula*, S3 was found to be most potential in inhibiting M1 bacterium giving the highest Zone of Inhibition value of 19.33mm at 0.5mg/100 µl and 21.00mm at 1mg/100 µl. M2 was among the most susceptible of all the microorganisms followed by M4, whose growth was effectively inhibited by all the extracts of this plant among which S1, S3 and S4 was found to be most effective. M3 was found resistant to all the extracts (S1-S4) of the plant. Though *T. chebula* extracts was effective against M1-M4 microbes, M5 was found showing resistant to all the extracts of this plant (Fig 1-4).

B. Determination of Minimal Inhibitory Concentration (MIC) and MBC

MIC and MBC values obtained for the extracts of *T.chebula*, revealed that S1 and S2 extract had the same MIC and MBC values and S3 and S4 extract gave the values at par with each other. MIC for M3 and M5 was not performed as the preliminary antimicrobial test showed no results against this bacterium. The least MIC value for M1 was exhibited by S3 and S4 extracts which was lesser than the positive control. Against M2 bacterium, all the extracts of *T. chebula* gave the same value of 0.125mg/ml. Whereas, M3 was potentially inhibited by S1 and S2 extracts having the MIC value of 0.0625mg/ml. MIC Index value was again same as other extracts..

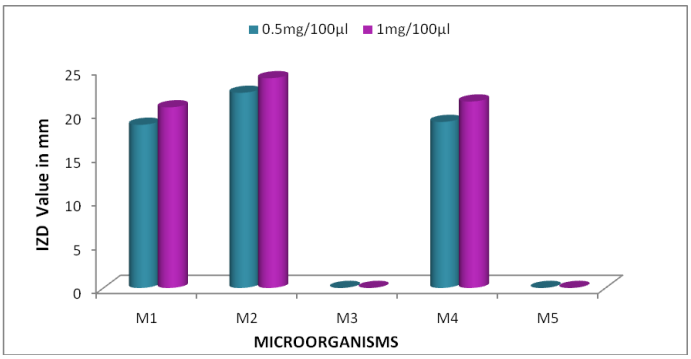

Fig. 1. Demonstration of IZD value (mm) of S1 extract of *T. chebula against Streptococcus mutans* (M1), *Streptococcus pneumonia* (M2), *Streptococcus pyogenes* (M3), *Staphylococcus aureus* (M5) and *Pseudomonas aeruginosa* (M5).

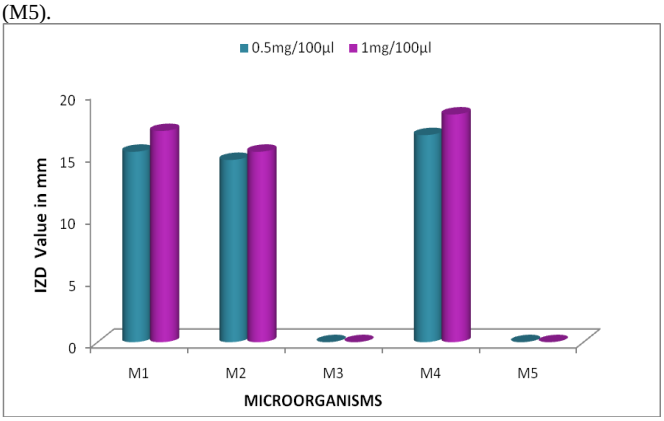

Fig. 2. Demonstration of IZD value (mm) of S2 extract of *T. chebula against Streptococcus mutans* (M1), *Streptococcus pneumonia* (M2), *Streptococcus pyogenes* (M3), *Staphylococcus aureus* (M5) and *Pseudomonas aeruginosa* (M5).

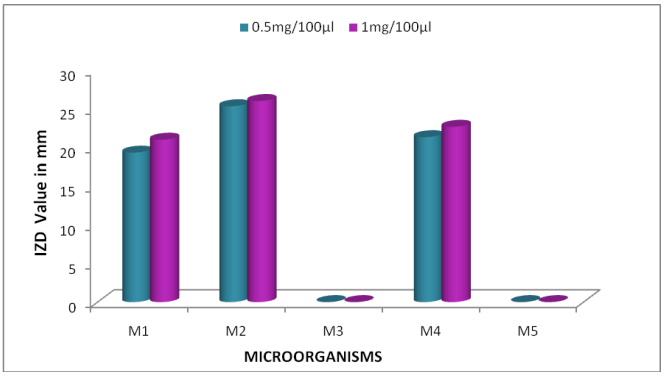

Fig. 3. Demonstration of IZD value (mm) of S3 extract of *T. chebula against Streptococcus mutans* (M1), *Streptococcus pneumonia* (M2), *Streptococcus pyogenes* (M3), *Staphylococcus aureus* (M5) and *Pseudomonas aeruginosa* (M5).

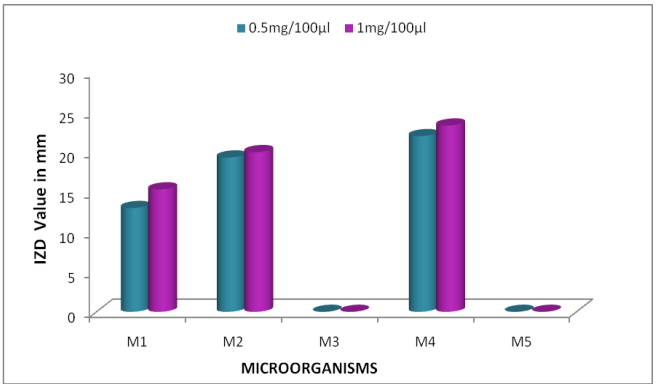

Fig. 4. Demonstration of IZD value (mm) of S4 extract of *T. chebula against Streptococcus mutans* (M1), *Streptococcus pneumonia* (M2), *Streptococcus pyogenes* (M3), *Staphylococcus aureus* (M5) and *Pseudomonas aeruginosa* (M5).

C. Determination of Antioxidant Activity of T. chebula Extracts

TABLE I: REPRESENTS THE ABSORBANCE AND ANTIOXIDANT POWER OF ETHYL ACETATE (S1), ACETONE (S2), METHANOL (S3) AND AQUEOUS (S4) EXTRACT OF *T. CHEBULA*

S.No	Concentration of the extract in g/l	Antioxidant Power of S1 extract in M/l	Antioxidant Power of S2 extract in M/l	Antioxidant Power of S3 extract in M/l	Antioxidant Power of S4 extract in M/l
1	6×10^{-4}	16.42	19.83	19.83	10.00
2	6×10^{-4}	26.33	19.83	27.42	24.75
3	6×10^{-4}	36.58	25.25	27.58	35.75
4	6×10^{-4}	42.83	32.58	32.17	42.58
5	6×10^{-4}	48.25	38.06	39.92	47.58
6	6×10^{-4}	53.75	43.17	48.25	50.67
7	6×10^{-4}	56.83	45.75	53.42	56.08
8	6×10^{-4}	59.33	50.75	56.08	58.83
9	6×10^{-4}	62.67	54.08	57.33	60.75
10	6×10^{-4}	63.33	55.25	58.17	60.83

The ability of *T. chebula* extracts to scavenge excess of Fe^{3+} ions or to convert them to more stable ions of Fe^{2+} exhibited a different result than its other species *T. bellirica*. The free radical scavenging property of the extracts of *T.chebula* were concentration dependent and showed a trend S1 > S4 > S3 > S2. At a lower concentration of the extracts S2 and S3 extracts exhibited better scavenging power. With increase in concentration the antioxidant power of S1 and S4 was better than S2 and S3, which was increasing with increase in their respective concentration but in comparison was lesser than the S1 and S4 extract. The results are recorded and represented as Table I.

V. DISCUSSION

A. Determination of Antimicrobial Property of T. chebula:

Bag, A. *et al.*, 2009[14] and Chaudhari, M. and Mengi, S., 2005[15] performed the antimicrobial activity on *Terminalia* species on various microorganisms to obtain the result which was similar to that found in our showing *Terminalia* species to possess antimicrobial property which also supports data obtained. The present study shows that all the extract of *T. chebula* possessed strain specific antimicrobial activity and S3 extract were found to be the most potential antimicrobial agent in inhibiting M2 microbe, S2 were found to possess the least potential antimicrobial activity giving moderate results as compared to other extracts. Similar results were obtained in case of M1 bacterium where this microbe was found to be susceptible to S3 extract Chebula extracts S1-S4 were ineffective in inhibiting M3 bacterial growth giving no IZD values. This study is comparable to previously reported results in literature where all the four microbes are effectively inhibited with acetone extract of the plants with variations in IZD values [16].

The result of MIC study on *T.chebula* by Bag, A. *et al.*, 2009 [14], reveals the significant inhibition of uropathogenic strains as well pure strains of pathogens by ethanol and aqueous extracts of *Chebulic* myrobalans. Our study shows better inhibitory concentrations of having a lower MIC and MBC values as compared to theirs‘ in inhibiting microbes causing various eye infections.

The Ferric-Reducing Antioxidant Power (FRAP) assay measures the antioxidant potentials of antioxidants to reduce the Fe3+/ 2,4,6-tripyidyl-s-triazine (TPTZ) complex present in stoichiometric excess to the blue coloured Fe2+ form. The study by various groups of researchers [9], [17] revealed the methanolic, aqueous and chloroform extracts of *Chebulic* myrobalans to possess antioxidant power to either enhance production of enzymes responsible for scavenging properties or scavenge the oxidants produced during different ailments. The data obtained through our study apparently coincides with the reported ones (though varied methodology was used) wherein the extracts S1-S4 were found to be potent ferric ions reducer, which causes oxidative stress. At even low concentrations of the extracts 6×10^{-4} g/l the antioxidant power of organic extracts like ethyl acetate and acetone were found to possess highest scavenging power as compared to methanolic and aqueous extracts. The antioxidant power was found to be concentration dependent. The free radical scavenging activity in the different extracts decreased in the following order: Ethyl Acetate > Aqueous > Methanol > Acetone.

VI. Conclusion

The antimicrobial and free radical scavenging study suggests that the plant extracts retained its ability to inhibit the growth of bacteria and antioxidant activities during the course of extraction and can counteract the oxidative damage induced by the pathogens or other means of oxidative stress responsible for various disorders. The phytochemicals of the plants were not compromised as they showed conclusive results in the study suggesting that the extraction procedure applied for obtaining the phytochemicals were optimum. The microbes screened are mostly involved in various pathogenic diseases in humans, *S.aureus* being the causative agent for bacteremia, and sepsis, *S.pneumoniae* acute sinusitis, meningitis, bacteremia, sepsis, arthritis, brain abscess, *S.pnemoniae* and *S.pyogenes* causes septicaemia and postoperative traumatic etc.

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