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Antimicrobial activity of aqueous extract of *Terminalia chebula* Retz.

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Abstract

Terminalia chebula belonging to the family Combretaceae is called the "king of medicines" in Tibet and used in the treatment of various diseases such as diabetes, depression, memory loss, cardiovascular diseases, leprosy etc and also inhibits the growth of malignant tumors. *T.chebula* fruit is rich in phytochemical constituents such as tannins, flavonoids and essential oils. In the present study antimicrobial activity of aqueous fruit extract of *T.chebula* was evaluated by Agar well diffusion method against gram +ve (*Bacillus sphaericus, Staphylococcus aureus), gram -ve (Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhimurium, Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli)* and fungal *(Candida albicans)* strains at different concentrations. Among the microbial strains evaluated *P.mirabilis and K.pneumoniae* were found to be the most sensitive, based on the results demonstrated from the inhibition zones. Thus, *T.chebula* fruit can be considered as a potential candidate for new antimicrobial drugs owing to its broad spectrum activity.

Keywords: Antibacterial, Antifungal, Microorganisms, Agar well diffusion, Antibiotic resistance.

INTRODUCTION

Medicinal plants are used for treating various diseases since prehistory (1). Plants are rich source of phytochemicals such as, phenols, flavonoids, essential oils etc which act as antimicrobials. Last few decades have witnessed the development of several synthetic drugs, however, due to the increase in the bacterial resistance towards the existing chemotherapeutics attention has been drawn for exploration of new natural antimicrobials (2). The main advantage of using plant extracts is that, the crude extracts contain a mixture of compounds viz. phenols, acids, esters, aldehydes etc., for which it is difficult to develop resistance by bacteria unlike the synthetic antibiotics that contain a single compound (3). The world health organization has taken an initiative for the development of plant based health care products and making them available to maximum number of people (4).

Terminalia chebula Retz, (family Combretaceae) is a flowering evergreen tree attaining a height up to 30m and widely distributed throughout India, Burma and Srilanka. The dried ripe fruit of *T. chebula* also known as black myrobalan has been widely used in the treatment of asthma, sore throat, vomiting, bleeding etc (5) and displayed strong antimicrobial activity against *Helicobactor pyroli* (6) and *Escherichia coli* (7). Black myrobalan has reported to have antioxidant and free redical scavenging activities (8). *T.chebula* fruit contains an active compound called Chebulic acid which is a phenolic acid (9). The present study is aimed to evaluate the antimicrobial potential of aqueous fruit extract of *Terminalia chebula* at different concentration.

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MATERIALS AND METHODS

Plant collection and Preparation of the sample

T. chebula fruits were collected locally from Farm wealth Bio-tech, Hyderabad. The fruits were dried and milled to fine powder and extracted with water. Filtered extract was lyophilized till drying. Lyophilisation enables concentration of plant components by evaporation under pressure and low temperature through lyophilizer. The lyophilized extract was stored at 4°C for further use. The extract at the concentration of 1mg was used for testing the activity.

Antimicrobial assay

The antimicrobial activity of the extract was determined using the Agar well diffusion method (10). Pour plate technique was used with an inoculum size of 10^6 colony forming units (cfu)/ml of bacteria. A borer with a diameter of 8mm was used for making the wells in the MHA plates. The extracts ranging from 500µg to 2000µg concentrations were dispensed into each of the wells. Amikacin, a broad spectrum antibiotic at a concentration of 50µg was used as the positive reference standard. Sterile distilled water was used as a negative control. After 24h incubation period at 37°C the zones of inhibition around each well were measured. By comparing the inhibitory zones around the well the sensitivity of the pathogens towards the extracts was determined. All the experiments were performed in triplicate and analyzed.

Microbial Cultures

Bacteriological media (Mueller Hinton Agar (MHA) & Mueller Hinton Broth (MHB)) were procured from Himedia. The microbial cultures- *Staphylococcus aureus* MTCC 7443, *Bacillus sphaericus* MTCC 511, *Escherichia coli* MTCC 7410, *Pseudomonas aeruginosa* MTCC 2295, *Proteus mirabilis* MTCC 425, *Proteus vulgaris* MTCC 744, *Salmonella typhimurium* MTCC 98, *Klebsiella pneumoniae*

MTCC 3384 and *Candida albicans* MTCC 3017 were obtained from IMTECH, Chandigarh. All the cultures were maintained on Mueller Hinton agar at 4°C and sub-cultured every fortnight and reactivated in Mueller Hinton broth before the antimicrobial assay.

Statistical analysis

Results were expressed as the mean ± standard deviation from the obtained triplicate data. The data was compared by least significant difference test using Statistical Analysis System (ver 9.1).

RESULTS AND DISCUSSION

Plants synthesize a wide variety of secondary metabolites as part of defence mechanism against attack by insects, herbivores and microorganisms (11). Due to the increase in the incidence of antibiotic resistance among microbe, attention has been drawn in search of new drugs from different sources. One such source is the plant biodiversity, as these green medicines are nontoxic, healthier and safe, in comparison to the synthetic drugs. *Terminalia chebula* fruit extracts showed good antimicrobial activity against oral bacterial strains (12). Phytochemistry and various pharmacological properties of *Terminalia chebula* has been studied which provide scope for evaluation of this plant as medicinal agent against the human diseases (13).

In the present study the phytochemical constituent of the aqueous fruit extract of *Terminalia chebula* were analysed. Clinically important microorganisms were used to assess the activity of extracts. The antimicrobial activity of aqueous fruit extract of *Terminalia chebula* against the different strains has been illustrated in Table 1. The aqueous extracts have shown good activity towards all the pathogens showing its broad spectrum nature (Fig. 1)

Table 1. Antimicrobial activity of aqueous fruit extract of *Terminalia chebula* Retz.

Micro-organisms	Concentration (µg)	Zone of inhibition of aqueous fruit extract (mm)	Antibiotic (50µg)
Gram positive			
Staphylococcus aureus	500 µg 1000 µg 1500 µg 2000 µg	11.5±0.34 13.5±0.17 15.66±0.15 18.33±0.15	31.23±0.15
Bacillus sphaericus	500 µg 1000 µg 1500 µg 2000 µg	13.56±0.35 14.8±0.17 15.66±0.40 18.33±0.15	36.33±0.4
Gram negative			
Escherichia coli	500 µg 1000 µg 1500 µg 2000 µg	13.46±0.30 15.33±0.41 16.4±0.26 19.88±0.11	34.53±0.32
Pseudomonas aeruginosa	500 µg 1000 µg 1500 µg 2000 µg	14.83±0.35 16.76±0.15 17.36±0.15 19.5±0.26	32.2±0.36
Salmonella typhimurium	500 µg 1000 µg 1500 µg 2000 µg	17.66±0.15 18.33±0.32 20.43±0.30 22.66±0.15	30.33±0.35
Proteus vulgaris	500 µg 1000 µg 1500 µg 2000 µg	16.33±0.36 17.8±0.55 19.7±0.62 21.9±0.55	30.36±0.35
Proteus mirabilis	500 µg 1000 µg 1500 µg 2000 µg	22.5±0.17 24.66±0.15 25.43±0.25 26.56±0.28	27.23±0.25
Klebsiella pneumoniae	500 µg 1000 µg 1500 µg 2000 µg	18.73±0.49 21.6±0.36 22.66±0.25 23.6±0.36	30.2±0.26
Fungal			
Candida albicans	500 µg 1000 µg 1500 µg 2000 µg	14.46±0.40 15.33±0.32 17.36±0.28 18.53±0.35	30.4±0.36

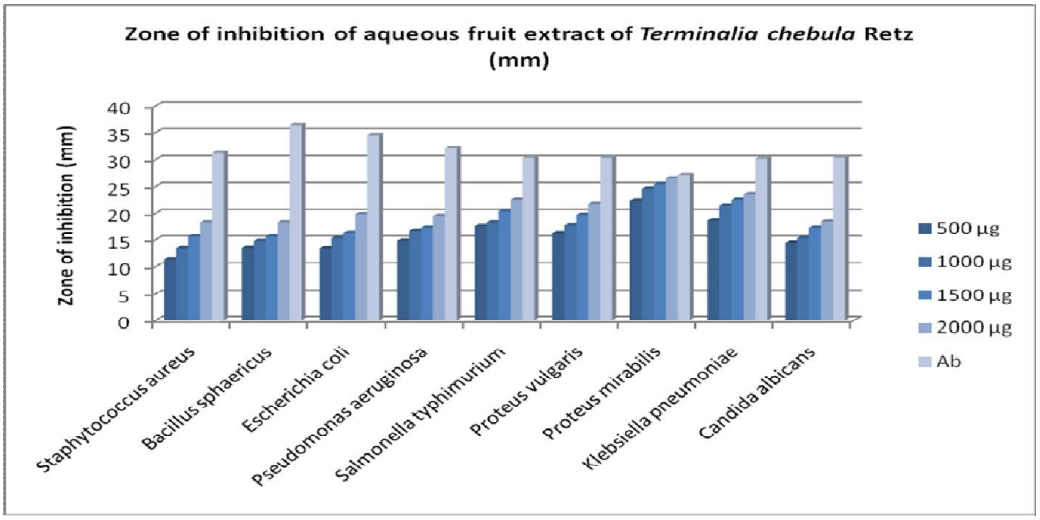

Fig 1. Antimicrobial activity of aqueous fruit extract of *Terminalia chebula* Retz

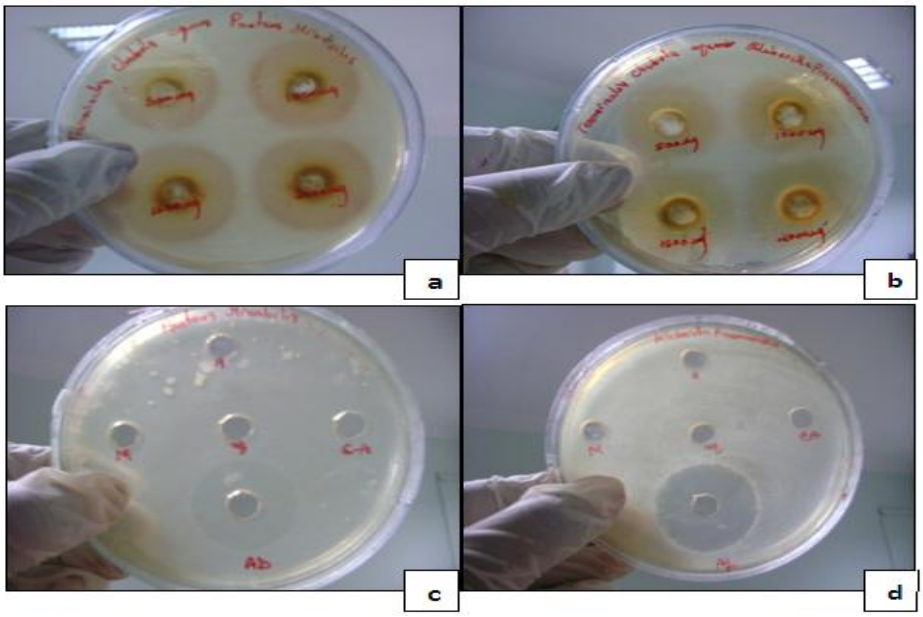

Fig 2. Zone of inhibition of aqueous fruit extract of *Terminalia chebula* Retz (a) *Proteus* mirabilis (b) *Klebsiella pneumoniae* (c) & (d) Zone of inhibition of antibiotic (50µg) against *Proteus mirabilis* and *Klebsiella pneumoniae.*

Amikacin served as the positive control. The inhibition zone of aqueous extract were in the range of 11.5±0.34mm - 18.33±0.15mm in gram +ve strains and 13.46±0.30mm - 26.56±0.28mm in gram negative strains and 14.46±0.40mm - 18.53±0.35mm in fungal strain. The highest zone of inhibition was obtained against *Proteus mirabilis* (26.56±0.28mm) (fig 2). The efficiency of the extracts can be seen from the zones of inhibition obtained against potent microbes like *K. pneumoniae* (23.6±0.36mm) and *S. typhimurium* (22.66±0.15mm). The most resistant organism in the present study was found to be *P. aeruginosa* (19.5±0.26mm). The permeability of the compounds and the resistance mechanisms displayed by the microbes could be the reason for the variable zones of inhibition exhibited by the organisms. In the previous reports the ethanolic extract of *T. chebula* fruit has demonstrated strong antimicrobial activity against multidrug resistant bacteria (14).

CONCLUSIONS

In the present study the aqueous fruit extract of *Terminalia chebula* has displayed good antimicrobial activity against gram positive, gram negative and fungal microorganisms, displaying its potential in the development of new phytopharmaceuticals. As these drugs are plant based, they can be considered safe for human. However, since the experiment conducted were based on crude extract further studies are needed in this direction.

REFERENCES

[1] Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin Microbiol Rev. 12 (4): 564-82.

[2] Gislene, G. F. N., L. Juliana, C. F. Paulo and L. S. Giuliana. 2000. Antibacterial activity of plant extracts and phytochemicals on

antibiotic resistant bacteria. Brazilian Journal of Microbiology. 31: 247–256.

[3] Kiranmayee, R., Ch. Bhuvaneswari, M. N. Lakshmi and G. Archana. 2010. Antibacterial activity of *Alpinia galanga* (L) Willd crude extracts. Appl. Biochem. Biotechnol. 162: 871–884.

[4] Goud, P. S. P., K. S. R. Murthy, T. Pillaiah and G. V. A. K . Babu. 2005. Screening for antimicrobial and antifungal activity of some medicinal plants of Nallamala in Andhra Pradesh. India. J Econ Taxon Bot. 29: 704–708.

[5] Anwesa, B., K. B. Subir, B. Premananda, K. P. Nishith and R. C. Rabi. 2009. Evaluation of antibacterial properties of Chebulic myrobalan (fruit of Terminalia chebula Retz.) extracts against methicillin resistant Staphylococcus aureus and trimethoprimsulphamethoxazole resistant uropathogenic Escherichia coli. Afr JPS. 3(2): 025-9.

[6] Malekzadeh, F., H. Ehsanifar, M. Shahamat, M. Levin and R. R. Colwell. 2001. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobacter pylori*, Int. J. Antimicrob. Agents. 18: 85-88.

[7] Chatopadhyay, R. R., S. K. Bhattacharyya, C. Medda, S. Chanda, S. Datta and N. K. Pal. 2007. Antibacterial activity of black myrobalan (Fruit of *Terminalia chebula* Retz) against uropathogen *Escherichia coli*. Phcog. Mag. 11: 212-15.

[8] Cheng, H. Y., T. C. Lin, K. H. Yu, C. M.Yang and C. C. Linn. 2003. Antioxidant and free redical screening activities of *Terminalia chebul*. Bio Pharm. Bull. 26: 1331-35.

[9] Lee, H. S., Y. C. Koo, H. J. Suh, K. Y. Kim and K. W. Lee. 2010. Preventive effects of chebulic acid isolated from *Terminalia chebula* on advanced glycation endproduct-induced endothelial cell dysfunction. Journal of Ethnopharmacology 131 (3): 567–574.

[10] Perez, C., M. Pauli and P. Bazevque. 1990. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis. 15:113–115.

[11] Pesewu, G. A., R. R.Cutler and D. P.Humber. 2008. Antimicrobial activity of plants used in traditional medicine of Ghana, with particular reference to MRSA. Journal of Ethnopharmacology. 116: 102–111

[12] Kamal R. A and J. Radhika. 2009. Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens. Jundishapur Journal of Microbiology. 2(3): 105-111.

[13] Anwesa, B., K. B. Subir and R. C. Rabi. 2013. The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. Asian Pacific Journal of Tropical Biomedicine.3(3): 244-252.

[14] Anwesa, B., K. B. Subir, K. P. Nishit and R. C. Rabi. 2012. Invitro antimicrobial potential of *Terminalia chebula* fruit extracts against multi drug- resistance uropathogens. Asian Pacific Journal of Tropical Biomedicine. (2012) 1-5.