

Efficacy of ethyl acetate and ether extract of *Terminalia chebula* Retz against some human pathogenic strains

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Abstract: The present investigation was undertaken to evaluate *in vitro* antimicrobial activity of *Terminalia chebula* Retz (Combretaceae). The crude ethyl acetate and ether extract of *T. chebula* (fruit and seed) was assayed for their antimicrobial activity using disc diffusion method. The activity was performed against common pathogenic bacterial (*Staphylococcus aureus*, *Proteus vulgaris* and *Escherichia coli*) and fungal strains (*Aspergillus niger* and *Candida albicans*). The extracts of *T. chebula* were found to be more or less active against almost all tested pathogenic strains. The inhibition zone ranged from 6 mm - 22 mm and activity index ranged from 0.18 – 1.25 mm. *S. aureus* and *A. niger* were the most susceptible bacteria and fungi respectively. The fruit extracts of *T. chebula* was found to be the most active that exhibited more or less similar activity against all the tested strains. However, among both the extracts the ethyl acetate exhibited comparatively higher activity. The significant potential of *Terminalia chebula* extract concludes that it could serve as a source of natural antimicrobial agents.

Key words: *Terminalia chebula* Retz, human pathogenic strains, ethyl acetate and ether extract of *Terminalia chebula*.

Introduction

Terminalia chebula Retz is a medium to large-sized tree belonging to the family Combretaceae. Commonly known as black myrobalan and haritaki, it is distributed in the tropical and sub-tropical region of India. *T. chebula* is routinely used in traditional systems of medicine to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections^{1,2}. *T. chebula* is reported to possess anticancerous, antioxidant, hypolipidemic, hepatoprotective, antidiabetic and immunomodulatory activity^{3,4,5}. The fruits from *T. chebula* appear to have evolved as complex antibiotic compounds that have shown activity against bacteria^{6,7,8} and virus like Herpes simplex virus type-1 and Cytomegalovirus⁹.

Recently, due to indiscriminate use of commercial antimicrobial drugs, multiple drug resistance human pathogens have developed^{10,11,12}.

This situation has provoked interest for searching new antimicrobials from natural sources. Plants have formed the basis of sophisticated traditional medicine systems that have given rise to some important drugs still in use today^{13,14}. Plant metabolites and plant based drugs appear to be one of the better alternatives as they are known to have minimal toxicity and cost-effective in contrast to synthetic agents. Numerous studies have been conducted with various plants, screening antimicrobial activity as well as for the discovery of novel antimicrobial chemotherapeutic agents^{15,16,17}. Therefore, in the present investigation, the antimicrobial potential of *T. chebula* fruit and seed extracts has been evaluated against common pathogens.

Material and methods

Plant material

Plants parts (fruits and seeds) of *T. chebula* were collected from Jabalpur district in Madhya Pradesh, India. The plants were identified and voucher specimens were conserved to the Herbarium (RUBL No: 20823), Department of Botany, University of Rajasthan, Jaipur, India.

Extraction

The plant materials viz., fruits and seeds of *T. chebula* were air dried and powdered using motor and pestle. The coarsely powdered materials were successively extracted with ethyl acetate and ether in Soxhlet apparatus for 24 hrs. The extracts were further evaluated for their antimicrobial activity against selected pathogenic strains.

Antibacterial Screening

Test microorganisms

The *In vitro* antimicrobial activity was evaluated against common pathogenic bacteria (*Staphylococcus aureus*, *Proteus vulgaris* and *Escherichia coli*) and fungal strains (*Aspergillus niger* and *Candida albicans*). All the tested microorganisms were obtained from SMS (Sawai Mansingh) Hospital, Jaipur, Rajasthan. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37°C for 24h while the fungal cultures were maintained on Potato Dextrose Agar slants and incubated at 27°C for 48h.

Antimicrobial activity

Antimicrobial assay of the acetate and ether extracts were performed against tested pathogenic strains by agar disc diffusion method¹⁸ using ampicillin and fluconazole as standard antibiotic. The nutrient agar plates and potato dextrose agar plates were seeded with suspension (10^6 cfu/ ml) of the bacterial and fungal strains vice- versa. The empty sterilized Whatmann No.1 filter paper disc (6mm) were impregnated with 10 μ l of extract diluted with two volumes of DMSO, dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (Pre diffusion time). The standard discs (6mm) impregnated with antibiotic ampicillin (2 μ g/ml) and fluconazole (2 μ g/ml) was used as positive control. The plates were incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi, respectively. The diameter of the inhibition zone (mm)

was measured and the activity index was also calculated.

Result and discussion

Medicinal plants have been considered a boon to human society to cure a number of ailments¹⁹. Plants have a great potential of producing natural drugs that have been the source of most of the active ingredients of medicines, as they are non-toxic, having no side-effects and easily available²⁰. Several works have been documented on the pharmacological screening of plant extracts which have been exploited as the source of innumerable therapeutic agents^{21,22,23}.

In the present investigation, the antimicrobial activity of crude ethyl acetate and ether extracts of *T. chebula* fruits and seeds were evaluated against some pathogenic strains. The activity was quantitatively assessed on the basis of inhibition zone and their activity index was also calculated (Table 1). *In vitro* antibacterial activity was determined by agar disc diffusion method using ampicillin and fluconazole as standard antibiotics. The extracts of *T. chebula* were found to be more or less active against almost all tested pathogenic strains. The inhibition zone ranged from 6 mm - 22 mm and activity index ranged from 0.18 – 1.25 mm. The most susceptible bacteria and fungi are *S. aureus* (IZ= 22mm and AI= 1.0) and *A. niger* (IZ= 11mm and AI= 0.34mm) respectively. The fruit extracts of *T. chebula* was found to be the most active that exhibited more or less similar activity against all the pathogens tested. However, among both the extracts the ethyl acetate extract exhibited comparatively higher activity. Lowest activity was noticed on *E. coli* (IZ= 8mm and AI= 0.50) and *A. niger* (IZ= 6mm and AI= 0.18) as compared to standard antibiotics.

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 membrane²⁴. 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^{25,26,27}. The findings of the present investigation validate their traditional use and suggests that *T. chebula* extracts has better efficacy and can be a source for natural antimicrobial agent.

Parts used	Microorganisms used						
			<i>S.aureus</i>	<i>P.valgaris</i>	<i>E.coli</i>	<i>A.niger</i>	<i>C.albicans</i>
Fruits	Ethyl acetate	IZ	18	15	20	08	09
		AI	0.81	0.35	1.25	0.25	0.28
	Ether	IZ	10	13	18	11	10
		AI	0.45	0.3	1.12	0.34	0.31
Seeds	Ethyl acetate	IZ	22	09	09	06	08
		AI	1.0	0.21	0.56	0.18	0.25
	Ether	IZ	22	08	08	11	08
		AI	1.0	0.19	0.50	0.34	0.25
Controls	Fluconazole	IZ	-	-	-	32	32
	Ampicillin	IZ	22	42	16	-	-

IZ= Inhibition Zone, AI= Activity index, - = not detected

Conclusion

The present investigation revealed that the extracts of *T. chebula* fruit and stem have potent antimicrobial activity which explains its use in traditional system of medicines. The extracts of *T. chebula* were found to be more or less active against almost all tested pathogenic strains. Hence, *T. chebula* can be employed as a source of natural antimicrobials that can serve as an alternative to conventional medicines.

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