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Original Research Article

Harad stem possesses antibacterial and antifungal efficacy

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Abstract

Introduction: Bacterial and fungal diseases devastate food crops like rice, wheat, maize, potato, *etc.* which could otherwise nourish those suffering from food insecurity. These crops constitute the primary source of calories ingested by individuals. Therefore, researchers seek novel antibacterial and antifungal chemicals from natural sources, which are less hazardous to human health and the environment.

Aim & Objective: The study aims to evaluate the antibacterial and antifungal properties of methanolic extract prepared from the stem part of *Terminalia chebula*, commonly known as harad, using *in vitro* methods.

Materials and Methods: The antibacterial efficacy was determined against two gram-positive and three gram-negative bacterial strains using the agar well diffusion assay, while the minimum inhibitory concentration (MIC) was determined through serial microdilution testing. Additionally, the antifungal activity against three phytopathogenic fungi was analyzed using the poisoned food technique, where varying concentrations of the extract were incorporated into sterilized potato dextrose agar (PDA) to measure fungal growth inhibition.

Results: The results demonstrated strongest antibacterial activity against *Escherichia coli*, exhibiting an inhibition zone of 17 ± 0.7 mm. In antifungal testing, the extract showed highest efficacy against *Bipolaris specifera*, with an IC_{50} value of 0.95 ± 0.01 mg/mL.

Conclusion: *Terminalia chebula* (harad) exhibits significant antimicrobial properties, likely attributable to its rich phytochemical composition. The methanol extract of harad stem shows particular promise for managing infections caused by the studied pathogens, thereby validating its traditional ethnomedicinal applications.

Keywords: Terminalia chebula, Harad, Antifungal, Antibacterial, Antimicrobial

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1. Introduction

Terminalia chebula (commonly known as Harad), a member of the Combretaceae family, contains numerous bioactive compounds including flavonoids, tannins, gallic acid, chebulinic acid, ellagic acid and punicalagin.[1] Previous studies have documented its diverse pharmacological properties, such as anti-oxidant,[2] antidiabetic,[3] anti-cancer,[4] anti-mutagenic,[5] anti-viral,[6] antibacteria[7,8] and radioprotective[9] effects. The antibacterial efficacy of an ethanol extract derived from *T. chebula* was evaluated against clinically relevant reference bacterial strains. Antimicrobial susceptibility was assessed using the disc diffusion method, while the minimum inhibitory concentration (MIC) was determined through broth microdilution assays. The extract demonstrated significant inhibitory activity against *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441, and *Pseudomonas aeruginosa* ATCC 27853. The MIC value for *S. typhi* was found to be 1 mg/mL. These findings suggest that *T. chebula* dry fruits exhibit broad-spectrum antimicrobial potential, warranting further investigation to identify the specific bioactive constituents responsible for this activity.[10] *In vitro* fungi toxicity of harad fruit pericarp was tested against three pathogenic moulds *viz.*, *F. solani* (seedling), *A. niger* (seeds) and *A. flavus* (seedling). The antifungal activity was determined by poisoned food technique and the MIC evaluated at three different concentrations. Methanol sample gave the best result with

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100% mortality of *A. flavus* and *F. solani* that demonstrated a new chemical utilization approach of the fruit pericarp of harad towards the development of biofungicides in the management of above fungi of common occurrence in Indian forestry.[11] In the present investigation, the stem part of the harad was evaluated against bacterial and fungal strains.

2. Materials and Methods

2.1. Plant material and preparation of the extract

The stems of *Terminalia chebula* were first identified and verified before collection, with adequate amounts of fresh stem material being gathered. The newly acquired stems were cut into smaller pieces, dried naturally in shaded conditions, and subsequently pulverized into a fine powder. To prepare the methanolic extract, 100 grams of the dried, powdered stem material was subjected to solvent extraction using pure methanol (99%), followed by evaporation to complete dryness under vacuum conditions.[12]

2.2. Determination of the antibacterial activity

The antibacterial properties of the test substance were qualitatively analyzed using a modified agar-well diffusion technique.[13] The study employed five bacterial strains: two Gram-positive (*Bacillus subtilis* MTCC 2389 and *Staphylococcus aureus* MTCC 7443) and three Gram-negative (*Micrococcus luteus* MTCC 4821, *Escherichia coli* MTCC 2127, and *Klebsiella pneumoniae* MTCC 7162). For the assay, 20 mL of sterilized nutrient agar was inoculated with 100 µL of bacterial suspension (10^8 CFU/mL) and dispensed into sterile Petri dishes. After solidification at ambient temperature, 6 mm diameter wells were created aseptically in the agar medium. Each well received 20 µL of the essential oil preparation (50% DMSO dilution). Control wells contained either chloramphenicol (10 µg) as positive control or DMSO alone as negative control. Following a 2-hour pre-incubation period at 4 ^{0}C to facilitate compound diffusion, the plates were incubated at 37 ^{0}C for 24 hours to observe inhibition zones.

2.3. Determination of MIC by broth dilution method

The broth microdilution method was employed to assess the minimum inhibitory concentration (MIC) of the test compound against selected bacterial strains. The experimental procedure involved preparing sterile nutrient broth (1 mL aliquots) in test tubes. The test extract, prepared as a DMSO (1:1) solution, was filtered through a 0.22 µm membrane filter prior to serial dilution in the broth to achieve final concentrations ranging from 62.5 to 2000 µg/mL. Each tube was inoculated with bacterial suspension to obtain a standardized inoculum density of 10^6 CFU/mL, followed by incubation at 37^{0}C for 24 hours. Appropriate controls were included: nutrient broth alone served as the positive control, while sterile broth without bacterial inoculation functioned as the negative control. Following incubation, bacterial growth was visualized by adding 50µL of p-iodonitrotetrazolium violet (INT) solution (0.2 mg/mL) to each test tube. The tubes were again incubated for 30 min at 37 ^{0}C. Development of pink colour in the tube (due to reduction of dye) indicated the bacterial growth whereas tubes without colour indicated no active bacterial growth. The minimum inhibitory concentration (MIC) was identified as the lowest test concentration that completely inhibited visible bacterial growth, as demonstrated by the absence of color change in the INT viability indicator. To ensure experimental reliability, all antimicrobial assays were conducted.

2.4. Determination of antifungal activity

The antifungal efficacy was assessed using the poisoned food technique, an agar dilution method, against three phytopathogenic fungi: *Alternaria alternata*, *Curvularia lunata*, and *Bipolaris specifera* (obtained from the Division of Plant Pathology, SKUAST-Jammu). Serial dilutions of the test compound were incorporated into sterilized potato dextrose agar (PDA), which was then dispensed into 90 mm Petri dishes. A 5 mm fungal plug (with mycelial surface facing downward) was aseptically transferred to the center of each agar plate. The plates were incubated at 26^{0}C, and radial hyphal growth was measured at 24-hour intervals until the control plates (without test compound) showed complete surface colonization. The percentage growth inhibition was calculated by comparing the fungal colony diameters in treated plates versus controls. All tests were performed in triplicate, with results reported as mean values of three independent replicates.

The antifungal indices were calculated as:
Antifungal index (%) = (1-Da/Db) ×100
Da = Diameter of growth zone in the experiment dish (mm)
Db = Diameter of growth zone in the control (mm)

Table 1: Antibacterial analysis of *Terminalia chebula* stem

Extract	**Conc. (mg/mL)**	**Bacterial strains**				
Methanolic	100 mg/mL (10µl)	*B. subtilis* MTCC 2389	*M. luteus* MTCC 4821	*S. aureus* MTCC 7443	*E. coli* MTCC 2127	*K. pneumoniae* MTCC 7162
		Zone of inhibition (mm)				
		11.6±0.3	10±0.26	13.6±0.35	**17±0.7**	12±0.4
MIC	62.5 µg/ml-2000 µg/ml	500	500	500	250	500

Positive control CMP^+	1mg/mL (10 µl)	36±1.3	35.6±1.2	26.2± 0.9	35±1.1	35±1.3
Negative control DMSO	10 µl	-	-	-	-	-

Bold numerals in the data tables denote the largest observed inhibition zones
DMSO: Dimethylsulfoxide; CMP^+: Chloramphenicol
Mark (-) indicates no activity

Table 2: Growth inhibitory effect of *Terminalia chebula* stem on fungal strains

Extract	**Conc. (mg/mL)**	**Phytopathogenic fungi**		
		Alternaria alternata	***Curvuleria lunata***	***Bipolaris specifera***
		Growth Inhibition (%)		
Methanolic	0.5	36	35	32
	1	45	43.75	56.25
	2	62.5	68.75	80
	IC_{50}	1.28±0.02	1.26 ±0.012	**0.95±0.01**
Amphotericin B **(positive control)**	**Conc. (µg/mL)**	**Growth Inhibition (%)**		
	10	48.5	46.20	50.75
	20	65.00	61.00	71.50
	40	83.60	81.60	85.69
	IC_{50}	9.5±0.1	12.1±0.4	5.7±0.2

Maximum growth inhibition by test material as indicated by IC_{50} value is emphasized through bold numbers

3. Results and Discussion

The methanolic extract of *T. chebula* stem was found more active against *E. coli* with zone of inhibition of 17±0.7 mm followed by *S. aureus* (13.6±0.35 mm), *K. pneumoniea* (12±0.4 mm), *B. subtilis* (11.6±0.3mm) and *M. luteus* (10±0.16 mm) (**Table 1**). The methanolic extract of stem part of harad showed growth inhibition of *B. specifera* fungal strain with IC_{50} values of 0.95± 0.01 mg/mL. However less activity was produced by the extract in case of *A. alternata* and *C. lunata* with IC_{50} values of 1.28 ± 0.02 and 1.26 ± 0.012 mg/mL respectively (**Table 2**). The theme of World Health Day 2011 was "*Antimicrobial Resistance: No Action Today, No Cure Tomorrow"* emphasized that development of resistance in microbes to antimicrobial agents is not a new problem, but one that is becoming more dangerous; serious efforts are needed to avoid regressing to the pre-antibiotic era. The World Health Organization (WHO) has emphasized urgent need for new antibiotics to combat the growing threat of antimicrobial resistance. Developing novel and more effective antibacterial and antifungal agents remains a critical research priority, particularly against pathogens that severely impact both human health and agriculture. Over 50% of modern clinical drugs originate from natural sources, many of which exhibit potent activity against infectious diseases and cancer. Plant-derived compounds, in particular, represent a valuable reservoir for discovering unique antimicrobial and anticancer agents.[14] In line with this objective, the present study evaluated the *in vitro* antimicrobial potential of a methanolic extract derived from the stem of *Terminalia chebula* (Harad). The antibacterial activity was assessed against two Gram-positive (*Bacillus subtilis* MTCC 2389, *Staphylococcus aureus* MTCC 7443) and three Gram-negative (*Micrococcus luteus* MTCC 4821, *Escherichia coli* MTCC 2127, *Klebsiella pneumoniae* MTCC 7162) bacterial strains using the agar well diffusion and serial microdilution (MIC) methods. The extract demonstrated significant antibacterial efficacy, with the highest zone of inhibition observed against *E. coli* (17 ± 0.7 mm), suggesting that *T. chebula* fruit may serve as a potential source for novel antibiotics. Additionally, the stem extract was screened for antifungal activity against three phytopathogenic fungi (*Alternaria alternata*, *Bipolaris specifera*, and *Curvularia lunata*) using the poisoned food technique. Varying concentrations of the extract were incorporated into sterilized potato dextrose agar (PDA), and fungal growth inhibition was measured. The results revealed notable antifungal activity against *B. specifera*, with an IC_{50} value of 0.95 ± 0.01 mg/mL, indicates strong inhibitory potential.

The widespread and often indiscriminate use of antibiotics has led to their frequent misuse, even for minor ailments. This practice has significantly contributed to the alarming decline in microbial susceptibility to existing antimicrobial agents. In response to this growing crisis, there is increasing scientific interest in exploring alternative therapeutic approaches, particularly plant-derived medicines. Natural products are gaining prominence as potential antimicrobial agents due to their perceived safety profile, reduced toxicity, and multi-target mechanisms of action compared to synthetic drugs. Consequently, the development of novel and more effective antimicrobial compounds from

natural sources has emerged as a critical research priority in the global fight against pathogenic microorganisms.[15]

4. Conclusion

The present study highlights the therapeutic potential of harad extracts in combating pathogenic bacteria and fungi that threaten human health. Furthermore, this plant-derived treatment offers a safe, potent, and cost-effective alternative for addressing infectious diseases in humans, livestock, and poultry.

5. Conflict of Interest

None.

6. Source of Funding

None.

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