



Antibacterial Activity of Ethanolic Extracts of *Terminalia coriacea* (Roxb.) Wight and Arn. Leaves against Foodborne Pathogens

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Pathogens spread on food items during the stages of storage, transit and processing. Thus, the eradication of these microorganisms holds significant importance. Ethanolic extract of *Terminalia coriacea* was used against *Escherichia coli* NCIM2134, *Pseudomonas aeruginosa* NCIM2037, *Klebsiella pneumonia* NCIM2706, *Bacillus subtilis* NCIM2920, *Staphylococcus aureus* NCIM5345 and *Staphylococcus epidermidis* NCIM5755 by disk diffusion and microdilution methods to evaluate the antibacterial activity. The ethanolic extract of *Terminalia coriacea* (Roxb.) leaves gave significant activity against all tested species of bacteria, which was compared to the standard disinfectant of 0.1% of commercial chlorhexidine. The inhibitions zones ranged from 6.45 ± 0.82 to 9.52 ± 0.30 for different bacterial strains. The minimum inhibitory concentration (MIC) was observed, ranging from 0.73 mg/mL to 1.35 mg/mL and the minimum bactericidal concentration (MBC) was observed, ranging from 0.73 mg/mL to 2.50 mg/mL. Based on the obtained results, it is suggested that the *T. coriacea* demonstrates antimicrobial properties against both Gram-positive and Gram-negative bacterial strains commonly encountered in food products. This plant extract can be further developed for the sanitizing of food items.

Keywords: Antibacterial activity, *Terminalia coriacea*, Chlorhexidine, Disk diffusion test, Microdilution.

INTRODUCTION

Phytoconstituents isolated from medicinal plants show toxicity to microorganisms through various modes [1]. Phytochemical evaluations of raw materials from medicinal plants include the screening, extraction, identification and isolation of the medicinally active compounds in plant materials [2]. Bioactive constituents obtained from the plants are flavonoids, alkaloids, glycosides, tannins, volatile oils, phenolic compounds, sterols and antioxidants [3]. Secondary metabolites may be biologically significant, like flavonoids or carotenoids, but they are not essential as nutrients [4]. A large group of the plants obtained compounds hypothesized to have disease-protecting properties [5]. Various phytoconstituents possess a broad variety of effects that may help in curing many diseases and disorders [6]. Phytoconstituents are vital in fighting diseases like diabetes [7], ulcers [8], piles, skin allergies [9], arthritis [10], asthma [11] and cancer [12]. Herbals are considered to have fewer side effects than other synthetic medicines [13]. These plant-

based formulations can cure diseases without having any adverse impact on human [14,15].

T. coriacea is an important medicinal plant that is obtained from India. It has been reported to have various activities like anticancer, anti-inflammatory, wound healing, antinociceptive and hepatoprotective properties [16]. Many species come under the *Terminalia* genus; among those, *T. coriacea* is a recently authenticated species for its medicinal properties [17]. It has rich in flavonoid content which support the various therapeutic effects such as hepatoprotective, wound healing and antinociceptive [18]. This plant is widely available in the dried and hot regions of Andhra, Telangana and Tamil Nadu states of India. Apart from India, it is also available in Thailand, Myanmar, Vietnam, Cambodia, Laos and Bangladesh.

EXPERIMENTAL

Plant material: The leaves of *T. coriacea* were collected from the Tirumala hillside in Tirupati city, India. The plant materials were authenticated by botanist Dr. Madhav Chetty

and the sample was preserved in the herbarium of the Botanical Survey of India (BSI), Attapur.

Extraction of raw materials: The raw material was extracted as per reported procedures with minor modifications [19]. The leaf powders underwent successive extraction using three distinct organic solvents, arranged in increasing order of polarity (petroleum ether > chloroform > ethanol > aqueous). The leave powder (300 g) was immersed in 1.5 L of petroleum ether individually and placed on an orbital shaker for 72 h. The decoctions were filtrated through Whatman filter paper No. 1. The remaining residue was again subjected to extraction using petroleum ether to isolate additional components, and subsequently combined with the filtrate. The entire residue was kept in air for drying and again extracted by chloroform, ethanol and water using the same method used for petroleum ether extraction. All the solvents were removed from the extracts using rota vapour. The extracts were stored in a water bath for several hours at 40 °C. The yield of each extract was measured after the solvent evaporation and then *T. coriacea* dry extracts were dissolved in 10% DMSO and stored at 4 °C for further studies [20].

Bacterial culture: A total of six strains viz. *Escherichia coli* NCIM2134, *Pseudomonas aeruginosa* NCIM2037, *Klebsiella pneumonia* NCIM2706, *Bacillus subtilis* NCIM2920, *Staphylococcus aureus* NCIM5345 and *Staphylococcus epidermidis* NCIM5755 as foodborne bacteria [21] were selected for the present studies. All these strains of bacteria were subcultured in a nutrient agar medium and incubated overnight at 37 °C. All the cultures were stored at 20 °C and used for screening.

Disk diffusion test: The antibacterial potentiality of the extracts was tested employing the disk diffusion method [22]. Inoculating Mueller-Hinton agar plates (pH 7.2) with the tested organism (made in a sterile saline tube) required back-and-forth streaking to ensure uniform spread. An ethanolic extract of 100 mL of *T. coriacea* leaves (at 100 mg/mL concentrations) were dispensed in wells. Both positive and negative controls consisting of 0.1% standard chlorhexidine and 10% DMSO, respectively were utilized in this experiment.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The MIC was tested in a sterile 96-well plate using the two-fold standard broth microdilution containing microorganisms of approx. 10^6 to 10^8 CFU/mL. Bacteria were sub-cultured for 24 h at 37 °C on Mueller-Hinton agar (pH 7.2). The maximum concentration of extract (5 mg/mL) was filled in 12 columns of a microtiter plate. The lowest concentration (0.009 mg/mL) was filled in 3 columns. Column 1 was taken as a positive control (only media, no bacteria and test extract), while column 2 was taken as a positive control (only media and bacteria). The minimum inhibitory concentration (MIC) is the ground concentration of test extracts capable of reducing visible growth. In contrast, the minimum bactericidal concentration (MBC) is the ground concentration of text extracts that can kill bacteria. The plates were incubated at 37 °C for 24 h until positive control growth was observed [23,24].

Statistical analysis: The study aimed to examine the variances in the inhibitory zone mean for a certain bacteria species,

as well as the variations in the susceptibility of the studied microorganisms. This was accomplished through the utilization of analysis of variance (ANOVA) and Tukey's posthoc tests at the significance level of $p < 0.05$. The utilization of descriptive statistics was employed to analyze the minimum inhibitory concentration (MIC) [25].

RESULTS AND DISCUSSION

The percentage yield and the characteristics of ethanolic extracts of *T. coriacea* leaves extract is presented in Table-1. Preliminary phytochemical screening for chemical constituents in ethanolic extracts of *T. coriacea* showed positive for the presence of alkaloids, saponins, tannins, steroids, terpenoids, phenols and anthraquinones, which may be the reason for their antibacterial activity [26,27].

Plant (material)	Macroscopic	Compactness	Yield (%)
<i>Terminalia coriacea</i> (Young leaves)	Brown/Dark	Semisolid	15.9%

Antibacterial activity: The antibacterial potentiality of ethanolic extract of *T. coriacea* leaves against the six microorganisms were studied. The establishment of zones of inhibition, minimum bactericidal concentrations (MBC) and minimum inhibitory concentration (MIC) were employed to investigate the antibacterial properties of plant leaf extracts (MIC). When the concentration of extracts increases, bacterial growth inhibition also increases. ANOVA was applied to examine the variability in the perceptivity of microorganisms against extracts ($p < 0.05$).

The inhibitory zone of *T. coriacea* leaves extract against foodborne microorganisms is given in Table-2. The inhibitory zone was between 6.45 ± 0.82 to 9.52 ± 0.30 mm. Outcomes revealed that the inhibitory zone of *T. coriacea* extracts was 7.12 ± 0.52 mm, 6.45 ± 0.82 mm, 8.16 ± 0.42 mm, 9.52 ± 0.30 mm, 9.45 ± 0.56 mm, 8.26 ± 0.65 mm on *E. coli*, *P. aeruginosa*, *K. pneumonia*, *B. subtilis*, *S. aureus* and *S. epidermidis* respectively. Thus due to high value of zone of inhibition, it is inferred that ethanolic extracts of *Terminalia coriacea* possessed tremendous antibacterial activity.

Strains	Zone of inhibition (mm)		
	<i>T. coriacea</i> extract	CHX	DMSO
<i>E. coli</i>	7.12 ± 0.52	9.12 ± 0.52	NA
<i>P. aeruginosa</i>	6.45 ± 0.82	11.00 ± 0.32	NA
<i>K. pneumonia</i>	8.16 ± 0.42	10.42 ± 0.30	NA
<i>B. subtilis</i>	9.52 ± 0.30	10.50 ± 0.15	NA
<i>S. aureus</i>	9.45 ± 0.56	8.00 ± 0.42	NA
<i>S. epidermidis</i>	8.26 ± 0.65	11.02 ± 0.58	NA

Each value represents Mean \pm SEM, compared to CHX group. One way ANOVA followed by Tukey's post hoc tests at a level of significance $p < 0.05$.

MIC and MBC determination: The result revealed in Table-3, *T. coriacea* leaves extract ascertained a broad-spectrum

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (MIC) AND MBC
(MINIMUM BACTERICIDAL CONCENTRATION) OF *T. coriacea*
EXTRACT AGAINST FOOD-BORNE PATHOGENS

Strains	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i>	1.35	2.50
<i>P. aeruginosa</i>	1.35	1.35
<i>K. pneumonia</i>	1.35	1.35
<i>B. subtilis</i>	1.15	1.35
<i>S. aureus</i>	0.73	0.73
<i>S. epidermidis</i>	0.73	1.35

effect against the studied six bacteria with raging MIC value from 0.73 to 1.35 mg/mL. Among those entries, *S. aureus* and *S. epidermidis* were giant susceptible bacteria with MIC values of 0.73 to 1.35 mg/mL. Results revealed that the MBC ranged from 0.73 to 2.50 mg/mL, whereas *S. aureus* gave the minimum MBC data 0.73 mg/mL.

In the disk diffusion process, it was observed that *B. subtilis* gave the highest zone of inhibition compared to other species. However *P. aeruginosa*, *E. coli* and *K. pneumonia* were found to be resistant against the extracts because of significantly less zone of inhibition. Usually, the exterior membrane of Gram-negative bacteria usually acts as a permeability barrier that permits only little hydrophilic molecules to enter the cell, not allowing certain antimicrobial agent molecules [28]. This different structure of Gram-negative bacteria made them more tolerant against foreign matter intake. The zone of inhibition of chlorhexidine against different bacterial strains was in the range of 9.12 ± 0.52 – 11.02 ± 0.58 mm. *S. aureus* and *S. epidermidis* were found to be huge susceptible microbes having a MIC value of 0.73 mg/mL. *S. aureus* also revealed lower MBC values of 0.73 mg/mL compared to other species. Furthermore, *E. coli*, *P. aeruginosa* and *K. pneumonia* had similar MBC and MIC values indicating that these bacteria can die and inhibit with the equivalent concentration of the ethanolic extracts of *T. coriacea* [29]. This finding supports the findings of Khan *et al.* [30] and Patel *et al.* [26]. The chemical constituents present in the leaves of *T. coriacea* have reason to have all antimicrobial activity.

Conclusion

The ethanolic extract of *T. coriacea* leaves exhibited the antimicrobial effects against six bacterial pathogens. The plant extracts exhibit potent antimicrobial properties that effectively hinder or even halt the proliferation of six different microbial populations belonging to various bacterial classifications. Consequently, these extracts hold potential as viable food additives and preservatives for regulating said microbial populations and safeguarding the well-being of both humans and animals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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