

ANTIMICROBIAL ACTIVITY OF *TERMINALIA BELLERICA*

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ABSTRACT

The antimicrobial activity of crude and methanol extract of *Terminalia bellerica* dry fruit was tested by disc diffusion method, against 9 human microbial pathogens. Crude aqueous extract of dry fruit at 4 mg concentration showed zone of inhibition ranging from 15.5-28.0 mm. *S. aureus* was found to be highly susceptible forming highest zone of inhibition, suggesting that *T. bellerica* was strongly inhibitory towards this organism. These pathogens were highly sensitive to the methanol extract forming 14.0 to 30.0 mm zone of inhibition suggesting that the methanol extract of *T. bellerica* was more effective than crude extract against most of the microbes tested except *E. coli* (enteropathogen) and *P. aeruginosa*. The minimal inhibitory concentrations (MICs) of crude and methanol extracts were determined by broth dilution technique which ranged from 300 to >2400 µg/ml and 250 µg to >2000 µg/ml respectively, indicating that *T. bellerica* was highly effective against *S. aureus* with lower MIC values. There were some biochemical alterations induced by *T. bellerica*. These results indicate that *T. bellerica* dry fruit possesses potential broad spectrum antimicrobial activity.

KEY WORDS

Antimicrobial activity, Biochemical alterations, Human pathogens, MIC, *T. bellerica*.

INTRODUCTION

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity (1, 2, 3, 4). *Terminalia bellerica* belongs to the family "Combretaceae". commonly known as belleric myrobalan. It is routinely used as traditional medicine by tribal folk of visakhapatnam district, to get remedies from several ailments such as fever, cough, diarrhea, skin diseases and oral thrush. Chemical substances of β-sitosterol, gallic acid, ethyle gallate, galloyl glucose, a new triterpene, the belleric acid and chebulagic acid have been isolated from fruits of *T. bellerica*. Fruit extract of *T. bellerica* produced fall in blood pressure of rats at a concentration of 70 mg/kg body weight (5). But reports on antimicrobial activity of *T. bellerica* were scanty, particularly on these strains of microorganisms and their biochemical processes. Therefore an attempt has been made to study the antimicrobial activity of the crude and methanol extract

of *T. bellerica* dry fruit on certain pathogenic microorganisms.

MATERIALS AND METHODS

Fresh *T. bellerica* dry fruits were purchased from Girijan Corporation of Visakhapatnam, Andhra Pradesh, India. The dry fruits, devoid of seeds were ground to a fine powder, mixed in sterile distilled water to give a concentration of 1 g/5 ml stock solution (pH: 6.9), which was stored in refrigerator until further use. The residual methanol extract was prepared by pulverizing 2 kg of dry fruits, which was mixed with 5 L of absolute methanol in an aspirator bottle for 48 h. Later the solution was collected and subjected to several cycles of distillation until a thick brown coloured paste was obtained. One gram of residual methanol extract (with zero% of methanol) was mixed in 5 ml of methanol to give a concentration of 1 µl= 0.2 mg of *T. bellerica*.

The microbial strains (6) used in this study were *Staphylococcus aureus* (ATCC 9144), *Streptococcus pneumoniae* (UTI isolate), *Salmonella typhi* (NCTC8393), *Salmonella typhimurium* (ATCC 23564), *Escherichia coli* (entero pathogen) *Escherichia coli* (uropathogen), *Pseudomonas aeruginosa* (ATCC25619), *Yersinia enterocolitica* (ATCC9610), and *Candida albicans* (ATCC 2091), procured from MTCC, Chandigarh, India. All chemicals, media components and antibiotic impregnated discs used in

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this study were procured from Hi Media, Mubai, India.

Disc diffusion method was performed as described earlier (6). Briefly, nutrient broth / or agar was used to culture bacteria and sabouraud's broth/ or agar was used to cultivate *C. albicans*. Fresh overnight cultures of inoculum (0.1 ml) of each culture containing 10⁸ cells, was spread on agar plate. Three sterile paper discs (5mm diameter) were placed in each agar plate and on two discs crude, methanol extract of each 4 mg in 20 µl volume was placed. On the third, 20 µl of absolute methanol was placed as a control. Ampicillin disc (10 µg) was placed as a positive control in all plates inoculated with bacteria and for *C. albicans* culture, nystatin (100 units/Disc) was used. The bacterial cultures were incubated at 37°C for 18-24 h and *C. albicans* at room temperature for 48 h. Zone of inhibition was measured. The microbes were plated in triplicates and average zone diameter was noted.

The minimal inhibitory concentrations were determined as described earlier (6). Different concentrations of *T. bellerica* crude extract in nutrient broth were serially diluted in duplicates. Control test tubes did not receive any *T. bellerica* extract. Later 10³ cells of *S. aureus* in 0.02 ml volume was added into each test tube and incubated at 37°C for 18-24 h. The lowest concentration of drug which inhibited the growth was considered as MIC. Similarly the MICs of crude and methanol extracts were determined against all other microorganisms.

The morphological and biochemical tests were performed according to Cappuccino and Sherman (7) as described earlier (8) in order to assess whether there are any medicinal plant extract induced morphological and biochemical alterations. The respective minimal inhibitory concentrations of *T. bellerica* were used for evaluation of biochemical tests and the controls were not treated with the plant extract. The morphological tests include, Colony morphology, Gram staining and determination of motility by hanging drop method. The biochemical tests performed were IMVic test (Indole, methyl red, Voges proskeur, citrate utilization test) to demonstrate organisms ability to secrete tryptophanase, enzymes involved in mixed acid fermentation, citrase etc), mannitol fermentation, Dnase test, urease, coagulase, catalase, oxidase, hydrolysis of starch, protein and lipids, and fermentation of various sugars by growing these organisms in various media in the presence and absence of plant extracts.

RESULTS AND DISCUSSION

The results were summarized in Tables 1-3. In the present study both crude and methanol extracts of *T. bellerica* were strongly inhibitory to *S. aureus*, forming large zone of inhibition (Table.1), i.e 28 and 30 mm respectively. The crude extract was less effective

Table 1. Antimicrobial activity of *T. bellerica* and Zone of inhibition in mm.

Organism	Crude extract	Methanol extract	Ampicillin
<i>S. aureus</i>	28.0	30.0	24.0
<i>S. pneumoniae</i>	25.0	28.0	20.0
<i>S. typhi</i>	16.0	20.0	14.0
<i>S. typhimurium</i>	20.0	24.0	12.0
<i>E. coli</i> (UTI)	20.0	28.0	0.0
<i>E. coli</i> (EP)	23.0	28.0	0.0
<i>P. aeruginosa</i>	20.0	14.0	0.0
<i>Y. enterocolitica</i>	15.5	17.0	18.0
<i>C. albicans</i>	19.5	21.0	20.0*

* Nistatin = 100 units/ disc. Absolute methanol control (20 µl/disc) showed zone diameters ranging from 0.0 to12.0 mm.

Table 2. Minimal inhibitory concentration of *T. bellerica* dry fruit

Organism	Crude extract µg/ ml	Methanol extract µg/ ml
<i>S. aureus</i>	300	250
<i>S. pneumoniae</i>	600	500
<i>S. typhi</i>	>2400	2000
<i>S. typhimurium</i>	1200	1000
<i>E. coli</i> (UTI)	2400	500
<i>E. coli</i> (EP)	600	>2000
<i>P. aeruginosa</i>	2400	2000
<i>Y. enterocolitica</i>	>2400	>2000
<i>C. albicans</i>	2400	2000

against *Y. enterocolitica* as it formed 15.5 mm zone of inhibition. The methanol extract (14 mm) was less effective against *P. aeruginosa* than the crude extract (20 mm) indicating that the active principle gainst this organism was present in crude extract. The lowest MIC values of crude and methanol extracts were against *S. aureus* (Table 2) suggesting that *T. bellerica* was most effective against *S. aureus* (Table 2). In the present investigation *T. bellerica* fruit extract produced morphological alterations where the actively motile organisms viz., *S. typhi*, *S. typhimurium*, *E. coli* and *P.*

Table 3. The morphological and biochemical alterations induced by crude extract of *T. bellerica* dry fruit

Test	SA		STM		ECU		ECE		YE		
	C	E	C	E	C	E	C	E	C	E	
Gram staining	+	+	-	-	-	-	-	-	-	-	-
Motility	-	-	+	±	+	+	+	+	+	±	
Indole	-	-	-	-	+	+	+	+	-	-	
Methyle red	+	-	+	-	+	+	+	+	-	-	
Mac Conkey agar	-	-	-	-	+	+	+	+	-	-	
Dnase	+	-	-	-	-	-	-	-	-	-	
Coagulase	+	-	-	-	-	-	-	-	-	-	
Mannitol salt agar	+	-	-	-	-	-	-	-	-	-	
Catalase	+	±	+	±	+	+	+	+	+	±	
Oxidase	-	-	-	-	-	-	-	-	+	-	
Urease	-	-	-	-	+	-	-	-	+	-	
Amylase	-	-	-	-	-	-	-	-	+	-	
Protease	-	-	-	-	-	-	+	+	+	-	
Lipase	+	-	-	-	-	-	-	-	+	-	
Fermentation of sugars	A,G	A,G	A, G	A, G	A, G	A, G	A,G	A,G	A,G	A,G	
Sucrose	NT	NT	++	--	++	++	++	++	--	--	
Glucose	NT	NT	++	--	++	++	++	++	++	--	
Mannitol	+-	--	NT	NT	++	++	++	++	--	--	
Dextrose	+-	--	NT	NT	++	++	++	++	--	--	

SA = *S. aureus*; STM = *S. typhimurium*; ECU = *E. coli* (uropathogen); ECE = *E. coli* (enteropathogen) ; YE = *Y. enterocolitica*; + = positive; - = negative; ± = faintly positive; NT = not tested A= acid formation; G = gas formation.

* The methanol extract also gave similar results.

aeruginosa have showed less motility. The biochemical alterations induced by these extracts were presented in Table 3. The Dnase enzyme activity was inhibited when these organisms were treated with *T. bellerica*, which was revealed by the absence of zone of degradation and pink color around the inoculum on toluidine blue Dnase agar medium. Coagulase is another major virulence factor of *S. aureus* which converts the host plasma fibrinogen to fibrin, forming blood clots. This enzyme was inhibited by this herbal extract which was confirmed when *S. aureus* grown in the presence of *T. bellerica* extract was unable to form coagulation of normal human plasma indicating either inactivation of coagulase enzyme or absence of protein product. It was observed that when a drop of control *S. aureus* culture was added to a mixture (a drop of normal saline, a drop of normal human plasma and one drop of fruit extract), the coagulation of plasma was arrested indicating that *T. bellerica*, naturally may act as an anti-coagulant. Similar results were obtained with *T. chebula* (sent for publication to else where), since both plants belong to the same family and fruits taste the same and possessed a few

similar phytochemical substances. This finding indirectly supports the results where the fruit extract of *T. bellerica* (70 mg/kg) produced fall in blood pressure of rats (5). Similarly there were alterations in several enzymatic reactions such as urease, tryptophanase, fermentation of sugars in these pathogens indicating that the active compounds of this fruit extracts might have interfered with the enzymes involved in those biochemical reactions. The inhibitory effect of fruit extracts of *T. bellerica* can be attributed to the chemical substances (gallic acid and ethyle gallate) that were present in the fruits (5). Tannins may also be present as in case of *T. chebula* fruits which possessed tannin-B (5). It has been recently reported that tannins and propyle gallate, were inhibitory to food-borne, water-borne and off-flavour producing microorganisms. The antimicrobial activity of propyle gallate was associated with the hydrolysis of ester linkage between gallic acid and polyols that occurs when the fruits ripe (9). Similar mode of action can be predicted for ethyle gallate of *T. bellerica* fruits.

The results of the present study differ from our previous results (6) where *Allium sativum* was found

to be less effective than *T. bellerica* against *S. aureus* and *S. typhimurium* since the MIC values of *A. sativum* were higher to these bacteria (8 and 7.5 mg/ml, respectively). These results indicate that *Withania somnifera* raw fruit extract was more effective than *T. bellerica* towards *Y. enterocolitica* forming 30 mm zone of inhibition at similar concentration (sent for publication). Methanol extract of *T. chebula* was more effective than *T. bellerica* at similar concentration where it formed 38 mm zone of inhibition against *S. aureus* (sent for publication). Recently it has been found that *T. chebula* at a dose of 200 mg/kg body weight improved the glucose tolerance by reducing 44% of reduction in the peak blood glucose at 2nd hour of glucose tolerance test (10). The antidyslipidemic and antioxidant activity of *Terminalia arjuna* stem bark has also been reported recently (11). It was evident from this study that *T. bellerica* fruit extract can be used against fever, superficial skin infections, urinary tract and diarrheal infections. These results clearly indicate that *T. bellerica* crude and methanol extracts of dry fruit possessed broad spectrum antimicrobial activity.

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