



IN VITRO SUSCEPTIBILITIES OF HUMAN BACTERIAL PATHOGENS ASSOCIATED WITH DACRYOCYSTITIS TO *TERMINALIA BELLERICA*

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ABSTRACT

Plants possess medicinal value because of the presence of bioactive constituents that produces a definitive physiological action on the human body. The present study was undertaken to investigate antimicrobial activity of *Terminalia bellerica* against various human pathogenic gram positive and gram negative bacterial strains causing dacryocystitis. The antibacterial activity was performed using qualitative agar well diffusion assay. The result indicated that the various extracts of selected plant exhibited antibacterial activity in which the highest was from ethanolic plant extract. Bacterial strain, *S. aureus* showed interesting susceptibility profile when evaluated using the selected plant extracts. Thus the results indicate the potential of the selected plant for further work on isolation and characterization of the active principle for antibacterial activity.

Key words: Antimicrobial activity, Dacryocystitis, Agar well diffusion assay, *T. bellerica*, Belleric myrobalan

INTRODUCTION

The increasing trend of using chemotherapeutic agents and antibiotics has led to its resistance exhibited by various pathogenic microbial agents.¹ As an alternative treatment medicinal plants are used to treat these infections caused by bacteria, virus, fungi and parasites. They are widely used because they are safe than synthetic alternatives, easily available, cheaper and possess no side effects.² Moreover, in many of the developing countries traditional medicine is one of the primary health care concern.^{3,4} Plants are commonly exploited in traditional medicine and their curative potentials are well documented.⁵ The medicinal plant '*Terminalia bellerica*' belongs to family 'Combretaceae' and commonly known as belleric myrobalan. It is routinely used as a traditional medicine to get rid of various ailments like fever, diarrhea, cough, skin diseases, oral thrush, ophthalmic disorders and is one of the basic constituent of an Ayurvedic preparation "Triphala".⁶⁻⁸ Different chemical substances like gallic acid, ethylene gallate, galloyl glucose, the belleric acid, chebulagic acid have been isolated from the fruits of *T. bellerica*.⁹ Reports on antimicrobial activity of *T. bellerica* were scanty, particularly on these isolated pathogenic bacterial strains causing dacryocystitis (infection of lacrimal sac which may lead to ocular disorders).

In view of its high medicinal potential in previous findings we tried to access antibacterial activity of the different extracts of *T. bellerica* dry fruit on certain bacterial isolates causing dacryocystitis.

MATERIALS AND METHODS

Fresh disease free fruits of *T. bellerica* were collected and were washed thoroughly several times with water and air dried in shade. The dry fruits devoid of seeds were grounded to fine powder and stored in polyethylene bag under refrigeration for further experimentation.

Extracts were prepared by soaking 5 g of the powdered dry fruit of the selected plant in 50 ml three solvents of different polarities; ether, ethanol and distilled water at ambient temperature for 48 hours on flask shaker. The etheric and alcoholic macerates were kept for 24 hours to evaporate the solvent. In the remaining residue, 50 ml of distilled water was added. All the macerates were then squeezed through double layered muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 10,000 rpm for 20 minutes. The supernatants were sterilized by passing through 0.2 μ disposable filters. The extracts (10%) thus obtained were used for the *in vitro* studies.

Bacterial pathogenic strains of *E. coli* (20 isolates), *Klebsiella pneumonia* (16 isolates) and *Pseudomonas aeruginosa* (25 isolates), *Streptococci pyogens* (15 isolates), *Staphylococcus aureus* (29 isolates) and *Staphylococci epidermidis* (17 isolates) causing dacryocystitis were isolated from the samples of the patients suffering from dacryocystitis. The purity of the culture was determined by morphological characters and biochemical test. The cultures were maintained at refrigeration temperature on Nutrient agar (HiMedia) slants and were sub cultured monthly and before use.

Antibacterial activity of the plant extracts was determined by agar well diffusion method.¹⁰ 20 ml of Muller Hinton agar (Hi Media) was poured in sterile petri dishes and was allowed to solidify. 10 ml of sterile, Muller Hinton agar medium (seed agar) was seeded with organisms (about 0.2 ml according to 0.5 Mc Farland's standard) was poured uniformly on the base. 8mm bores were made and 0.1 ml of the different extracts was added to respective bore. Streptomycin (100 μ g/ 0.1 ml) was used as reference standard to determine the sensitivity of the bacterial species tested. The plates were incubated at 37^o C for 24 hours and zone of inhibition if any were measured in mm (millimeter).

RESULTS AND DISCUSSION

The results of antibacterial activity of *T. bellerica* against pathogenic bacterial strains were summarized in Table 1. In the present study all the extracts were able to show inhibitory action against the test cultures. The inhibitory action of these plant extracts may be due to presence of active antimicrobial compounds present in it. The pathogenic *Klebsiella* species isolates were sensitive to ethanolic extract whereas, it was resistant to all other extracts. The reason may be that, it needs higher concentration to inhibit it or the organisms may have developed resistance. The most susceptible bacteria were *S. aureus* forming large zone of inhibition. The basis of varying degree of sensitivity of the test organisms may be due to the intrinsic tolerance of micro organisms and the nature and combinations of phytochemicals present in crude extract. The stronger extraction capacity of water and ethanol may have produced greater active constituents responsible for antibacterial activity. The ether (non polar) extracts were less effective than aqueous (polar) and ethanolic (semi polar) extract. All the plant extracts were more potent by inhibiting gram positive bacteria than gram negative bacteria. These observations are likely to be the result of the differences in cell wall structure between gram positive and gram negative bacteria, with gram negative outer membrane acting as a barrier to many environmental substances and antibiotics.¹¹

It was assumed that the data are sampled from the population with identical standard deviation (null hypothesis). From the results

statistical analysis our hypothesis was accepted. Thus the selected plant extracts were as effective as standard on the test organism (Table 2).

Table 1: Antibacterial activity of *t. Bellerica* on the pathogenic bacteria causing dacryocystiti

Name of the organisms	Mean zone of inhibition (mm)			
	Ether	Aqueous	Ethanol	Streptomycin
<i>E. coli</i> (20 isolates)	10	17	19	14
<i>P. aeruginosa</i> (25 isolates)	11	20	14	12
<i>Klebsiella</i> spp (16 isolates)	5	6	10	12
Coagulase negative Staphylococci (17 isolates)	18	19	19	16
<i>Staphylococci aureus</i> (29 isolates)	18	21	21	16
<i>Streptococci</i> spp (15 isolates)	7	16	18	15

Table 2: Dunnet multiple comparison test of antibacterial activity of *T. Bellerica*

Comparison	Mean difference	q*	p Value	Result
Standard Vs Ether extracts	2.667	1.035	P>0.05	Not significant
Standard Vs Aqueous extracts	-2.333	0.9052	P>0.05	Not significant
Standard Vs Ethanol extracts	-2.667	1.035	P>0.05	Not significant

* If the value of q is greater than 2.540 then p value is less than 0.05

STATISTICAL ANALYSIS

Statistical analysis was performed using Graphpad Instat software Version 3. Analysis of variance (ANOVA) was performed to determine statistically significant differences amongst plant extracts and the conventional antibiotics. Dunnet multiple comparison test was determined between the plant extracts and standard antibiotics

CONCLUSION

The present study reveals that *T. bellerica* possess antibacterial principle. However, further investigation on isolation and characterization of active principle of plant extract responsible for antibacterial activity is needed and it would give evidence or bioactive potential of medicinal plants.

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