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In vitro antibacterial activity of *Terminalia chebula* leaf gall extracts against some human pathogenic strains

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ABSTRACT

The leaf galls of *Terminalia chebula* is used widely as Karkatasringi in south Indian markets. Karkatashringi is an important crude drug employed in various indigenous systems of medicine against several diseases and the drug has diverse medicinal properties. The present study was carried out to understand the antimicrobial activity of various extracts. The antibacterial activity of *T. chebula* (leaf gall) was evaluated against ten bacterial strains including Gram-positive and Gram-negative bacteria using the agar-well diffusion method. Among the two extracts tested, the ethanol extract presented the best results against all the bacteria while aqueous extract showed moderate inhibition of the microbial growth. Each extract is unique against different microorganisms; *Staphylococcus aureus* was more susceptible to both extract among the tested organisms, whereas *Serratia marcescens* and *Proteus mirabilis* were less susceptible for ethanol and aqueous extract respectively. The inhibitory effect of the extracts was compared with standard antibiotic Ciprofloxacin.

Key Words: *Terminalia chebula*, leaf gall, antibacterial activity, agar well diffusion method.

INTRODUCTION

In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very important source for discovery of new agents for treating various ailments related to bacterial infections (Roopashree *et al.*, 2008). *Terminalia chebula* Retz. (Combretaceae), Commonly known as black myrobalan and haritaki, is an important plant used in indigenous systems of medicine as remedy for fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections (Kirtikar and Basu, 1987; Anonymous, 1999). *T. chebula* has been reported to possess anti-oxidant (Cheng *et al.*, 2003), anti-diabetic (Sabu and Kuttan, 2002), anti-cancer (Saleem *et al.*, 2002), anti-mutagenic (Kaur *et al.*, 2002), anti-viral (Ahn *et al.*, 2002), anti-bacterial (Kim *et al.*, 2006; Chat-

topadhyay *et al.*, 2007, Bag *et al.*, 2009) and radioprotective activity (Gandhi and Nayyar, 2005).

The leaf galls of *T. chebula* are used as a substitute for Karkatashringi, which is widely used in Ayurvedic and other traditional systems of medication. Karkatashringi is the main component of Karkatadi churna, Balabhadra churna, Sringadi churna which are used in treatment of asthma, tuberculosis, indigestion, heart diseases, fevers and liver disorders. Similarly, in Siddha system of medication, they are used in the preparation of Karisalai lehyam, Venpocesunai nei, Gana thailum for treating diseases like cough, bronchial asthma, diarrhea, dysentery (Shantha *et al.*, 1991).

The present study was undertaken to investigate the antibacterial activity of *T. chebula* leaf gall extract against ten human pathogens (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Serratia marcescens*, *Proteus mirabilis*, *Staphylococcus aureus*, *Staphylococcus citreus*, *Bacillus polymyxa*, *Bacillus cereus*).

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MATERIALS AND METHODS

Plant Material

T. chebula leaf galls were purchased from a local market of Bangalore, Karnataka (Southern India) in May 2010 and authenticated by Dr. S. Sundara Rajan and voucher specimen (JU-RUV-52) were deposited at the Research Unit in Vrکشayurveda, Jain University, Bangalore.

Preparation of Extract

One hundred gram of pulverized plant material were taken in five hundred capacity thimble of Soxhlet apparatus and refluxed with ethanol and water separately until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint colour. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). A portion of the residue was used for the antibacterial assay.

Bacterial Culture

The bacterial strains used in this study were clinical isolates. The isolates were identified by a standard method (Cowan *et al.*, 1993). The organisms were maintained on nutrient agar slope at 4°C and sub-cultured into nutrient broth by a picking-off technique (Aneja, 2003) for 24 hrs before use.

Bacterial Susceptibility Testing

In vitro antibacterial activity of the crude extracts was studied against Gram-negative and Gram-positive bacteria by the agar well diffusion method (Nair *et al.*, 2005). Nutrient agar (Hi Media, India) was used as the bacteriological medium. The extracts were dissolved in 10% aqueous dimethyl-sulfoxide (DMSO) to a final concentration of 100mg/ml. Pure DMSO was taken as the negative control and 50 mg/ml Ciprofloxacin as the positive control. 100µl of inoculum was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculum. Wells were prepared in the agar plates using a sterile cork borer of 6.0mm diameter. 100µl of test and control compound was introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35°C and examined after 24 hours (Ali-Shtayeh *et al.*, 1998; Colins *et al.*, 1989). The diameter of the zone of inhibition produced by each

Table 1: Antibacterial activity of *Terminalia chebula* leaf gall extract against bacterial strains.

	Ethanol extract	Aqueous extract	Ciprofloxacin
<i>P. aeruginosa</i>	9.27±0.82*	6.73±0.41**	13.33 ±0.67
<i>K. pneumonia</i>	10.00±0.58**	7.27±0.18**	14.67±0.67
<i>S. typhi</i>	9.00±0.58*	6.27 ±0.48**	12.00±0.58
<i>E. coli</i>	12.00±0.58*	9.33 ±0.88**	15.33±0.67
<i>S. marcescens</i>	5.93±0.66**	4.67±0.67**	11.33±0.33
<i>P. mirabilis</i>	8.00±0.58*	4.33±0.88**	11.67±0.33
<i>S. aureus</i>	19.07±0.64**	13.33±0.44**	24.00±0.12
<i>S. citreus</i>	12.27±0.48**	9.80±0.95**	20.67±0.18
<i>B. polymyxa</i>	14.00 ±0.58**	11.00±0.58**	21.87±0.47
<i>B. cereus</i>	12.60±0.50**	10.00±0.58**	21.33±0.18

The values are the mean of three experiments ± S.E.

* P<0.05, ** P<0.01 compared to standard

agent were measured with a ruler and compared with those produced by the commercial antibiotic Ciprofloxacin.

Statistical Treatments

The results of the experiment are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple pairwise comparison tests to assess the statistical significance. P≤0.05 was considered as statistically significant, using software ezANOVA ver. 0.98.

RESULTS AND DISCUSSION

Terminalia chebula is a well known plant used in ayurvedic traditional medicine for their effectiveness against wide range of diseases due to the advantage of the diversity of secondary metabolites responsible for their curing property. Despite the existence of potent antibacterial agents, the appearance of resistant or multi-resistant strains imposes the need for a permanent search and development of new drugs (Bastert, 2001).

The antibacterial activity of the crude extracts of *T. chebula* leaf gall was determined against 10 strains which include Gram-negative and Gram-positive bacteria (Table 1). The leaf gall extracts differ significantly in their activity. The ethanol extract exhibited highest antibacterial activity in terms of zone of inhibition against all the bacteria tested when compared to aqueous extract. This indicates that ethanol extract are better as compared to

aqueous extract. These observations may be attributed to two reasons; firstly, due to the nature of biologically active components (alkaloids, flavonoids, sterols, quinine, tannins etc.) which might be enhanced in the presence of ethanol (Kabir *et al.*, 2005). It has been documented that alkaloids, flavonoids and tannins are plants metabolites well known for their antimicrobial activity (Tschesche, 1971). Secondly, the stronger extraction capacity of ethanol could have produced a greater number of active constituents responsible for antibacterial activity. *Staphylococcus aureus* being the most susceptible organism, the ethanol extract could be more effective in infections related to *S. aureus* rather than other bacterial infections (Patel and Patel, 1957; Uday, 1877). Ciprofloxacin, which was used as a positive experimental control against all bacterial strains assayed, produced a good zone of inhibition, while no inhibitory effect could be observed for DMSO used as negative control.

T. chebula leaf galls have demonstrated antibacterial activity against clinical strains of selected microorganisms. The crude extract shows activity profile. As the crude extract is mixture of several constituents, it exerts better activity profile. The basis of varying degree of sensitivity of test organism is due to the intrinsic tolerance of microorganism, chemical nature and structure of the constituent for the mode of action on the control of growth of microorganism is beneficial. The plant has been used in curing various ailments in India; hence the phytoconstituents is useful to develop the molecules against infectious diseases. *T. chebula* leaf galls has shown the better activity profile against gram positive and gram negative bacteria especially against *S. aureus*.

CONCLUSION

The results of the investigation do not reveal which chemical compound is responsible for the aforementioned activity. Further studies are needed to isolate the exact active compound, which are responsible for the antimicrobial activity. In addition, *in vivo* studies are necessary to determine the toxicity of the active constituents, their side effects and diffusion in different body sites. Further research is required for the development of broad spectrum antibacterial agent of plant origin.

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