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Antibacterial Activity of Leaf Extracts of *Adhatoda vasica*

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ABSTRACT

The present study aimed to evaluate the *in vitro* antimicrobial activity of pharmacologically important *Adhatoda vasica* plant extracts against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella typhi*. The agar well diffusion method was adopted to determine antibacterial activity against all the tested microorganisms. The selection of extracts was based on a phytochemical screening for the presence of secondary metabolites. The methanolic extract of *A. vasica* showed a maximum zone of inhibition (18.17 ± 0.44 mm) for *S. aureus* and was effective against all bacterial strains tested, thus showing antimicrobial activity. The methanolic extract of the leaves was stronger than extracts based on other solvents such as chloroform and hexane, which showed moderate to weak activity, respectively.

Keywords: agar well diffusion, *in vitro*, methanolic extract, medicinal plants

INTRODUCTION

Many higher plants accumulate extractable organic substances in sufficient quantities to economically manage diseases. The plant compounds to treat infections are an age-old practice in developing countries where there is dependence on traditional medicine for a variety of diseases (Shibata *et al*. 2005; Pieboji *et al*. 2006). Plants have been a rich source of medicines because they produce a wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. In recent times, the rapid development of multi-resistant bacterial and fungal strains of clinically important pathogens has stimulated scientists to develop newer broad spectrum antimicrobial agents (Rojas *et al*. 2003). Antibiotic resistance has become a global concern (Westh *et al*. 2004). The clinical efficacy of many antibiotics in existence is being threatened by the emergence of multi drug-resistant pathogens (Bandow *et al*. 2003). Infectious diseases are the world's leading cause of premature deaths, killings almost 50,000 people every day (Singh *et al*. 2010). In recent years, drug resistance to human pathogenic bacteria is commonly reported all over the world (Huebner *et al.* 1998; Schjørring and Krogfelt 2011). Presently, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases, but are also often with adulterations and side effects (Babayil *et al*. 2004). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Lewis and Ausubel 2006). Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Abu-Sha-nab *et al.* 2004; Shiota *et al.* 2004). Throughout the history of mankind, many infectious diseases have been treated with herbal remedies and researchers are increasingly turning their attention to folk medicine. Continuous search leads to the development of better drugs against microbial infections (Benkeblia 2004).

Vasaka, also called Malabar nut tree, is well known throughout India. It is tall, with several branches, is dense and is an evergreen shrub. In Ayurvedic medicine, *Adhatoda vasica* has been used for a multitude of disorders including bronchitis, leprosy, blood disorders, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, sore-eye, fever and gonorrhea. It is useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders. A decoction of Vasaka leaves may be used to help with cough and other symptoms of colds. The juice of Vasaka leaves softens the bronchial tube. It is also useful in reducing aggravation of pitta and discomfort due to jaundice. The roots and bark possess a virtue well-known for their expectorant properties (Sampath *et al*. 2010). It has also been used to speed delivery during childbirth. Anticestodal activity of *A. vasica* extracts was tested against *Hymenolepis diminuta* infections in rats (Yadav and Tangpu 2008). The leaves of the plant were investigated for anti-ulcer activity using two ulcer models: ethanol induced and pylorus ligation plus aspirin-induced models (Shrivastava *et al.* 2006). Antioxidant and antiinflammatory activity was attributed to an isolated compound, vasicine (Srinivasarao *et al.* 2006).

The current investigation aimed to explore the antibacterial potential of different extracts of *A. vasica*.

MATERIALS AND METHODS

Plant material and preparation of the extract

Fresh leaves of *A. vasica*, free from disease, were collected from local areas in Davanagere district, Karnataka. The plant leaves were shade dried and then powdered using a mechanical grinder. 100 g of pulverized leaf material was soaked in 500 ml of hexane, chloroform and methanol (LR grade, Merck, India) using Soxhlet apparatus. Extracts were filtered under vacuum through a single sheet of Whatman No. 1 filter paper and the process was repeated until all soluble compounds had been extracted. The filtrate obtained was concentrated *in vacuo* using a Rotavapor (Buchi Flawil, Switzerland). The residue fraction was subjected to antimicrobial activity assays.

Phytochemical screening

The preliminary phytochemical analysis of chloroform, hexane and methanol extracts were carried out using previously described

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Short Communication

Table 1 Phytochemical screening of *A. vasica* constituents.

Extract	Alkaloids	Flavanoids	Triterpenoids	Sterols	Tannins	Saponins	Glycosides
ME	+	+	-	+	-	+	+
CF	-	+	-	-	+	-	+
HX	-	+	-	-	+	-	+

Abbreviations: HX - hexane extract; CF - chloroform extract; ME - methanol extract. Phytochemical test: '-' negative and '+' positive

Table 2 Antibacterial activity of *A. vasica* against clinically isolated strains (inhibition zone in mm).

Plant extract	Bacterial species			
	Pseudomonas aeruginosa	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Salmonella typhi*
Methanol extract	17.17 ± 0.44	17.00 ± 0.29*	18.17 ± 0.44*	17.50 ± 0.76*
Chloroform extract	12.83 ± 0.70	12.33 ± 0.43	14.13 ± 0.18	13.16 ± 0.67
Hexane extract	11.13 ± 0.83	10.38 ± 0.18	11.88 ± 0.56	11.50 ± 0.39
Ciprofloxacin	23.83 ± 0.73	23.17 ± 0.60	24.83 ± 0.44	21.90 ± 0.59

The values are the mean of three experiments ± S.E.
*$P < 0.001$ significant, when compared to control

methods (Harborne 1984; Trease and Evans 1989; Kokate *et al*. 1998; Khandelwal 2005). The dry weight of the methanolic extracts was obtained by allowing the solvent to evaporate and was used to determine concentration in mg/mL (Betoni *et al*. 2006).

Microorganisms used

Stock cultures of Gram-negative organisms (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*) and Gram-positive organism *Staphylococcus aureus* were obtained and confirmed at the research laboratory of Department of Microbiology, K.S. Hegde Medical Academy Mangalore, Karnataka. The bacterial strains used in this study are clinical isolates from different infection status of patients presenting symptoms of *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *S. typhi* associated diseases. The bacteria were maintained on a nutrient agar (Himedia, Mumbai) slope at 4°C and sub-cultured into nutrient broth by a picking-off technique (Aneja 2003). 24-h-old pure cultures were prepared for use each time.

Preparation of culture medium and inoculation

Nutrient agar was used as the bacteriological medium. The media was sterilized by autoclaving at 120°C for 20 min. Under aseptic conditions, in the laminar air flow, 15 ml of culture medium was dispensed into pre-sterilized Petri dishes to yield a uniform depth of 4 mm. After solidification of the medium, the microbial cultures were inoculated by spread plating technique.

Agar well diffusion assay

The extracts of the plants were screened for the antibacterial activity by the agar well diffusion method (Nair *et al*. 2005). Extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100 mg/ml. Pure DMSO was used as the negative control and 50 mg/ml Ciprofloxacin (Cipla, Bangalore) as the positive control. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter into which 10 mg/100 µL/well of each extract were loaded. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar and then they were incubated at 37°C for 18 h. Subsequently, the plates were examined for microbial growth inhibition and the inhibition zone diameter (IZD) measured in mm.

Statistical analysis

The results of the antibacterial study are expressed as mean ± SEM of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple pair wise comparison tests to assess the statistical significance. The data were considered significant at $P < 0.001$.

RESULTS AND DISCUSSION

The phytochemical profile of various solvent extracts from plant used in this study is presented in **Table 1**, although the dry weight of methanolic extract obtained was 75 mg/ml. The analysis revealed the presence of alkaloids, flavonoids, triterpenoids, sterols, tannins and glycosides. In particular, the methanolic extract tested positive for most of the secondary metabolites tested. Hexane and chloroform extracts showed the presence of flavanoids, tannins and glycosides. The leaf methanolic extracts were hence chosen for further evaluation of antibacterial activity. The microbial evaluation of the plant extract of *A. vasica* showed potent antibacterial activity compared to the standard antibiotic ciprofloxacin (**Table 2**). Among all the bacterial strains used, *S. aureus* was most susceptible to the methanol extract while *K. pneumoniae* was least susceptible.

Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Yao *et al*. 1995; Betoni *et al*. 2006). It has already been established that crude extracts of some medicinal plants and some pure compounds from such plants can potentiate the activity of antibiotics *in vitro* (Ozcelik 1998; Tortora *et al*. 2001). This search for antibiotic resistance modulators in plants represents a new dimension to addressing the problem of antibiotic resistance. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Shibata *et al*. 2005). In the present work, *A. vasica* extract used were extracted from polar solvent and hence had exhibited appreciable activity against clinical strains. Our findings are in agreement with reports showing that polar extracts inhibited the growth of both Gram-positive and Gram-negative bacteria in extracts of *Combretum caffrum*, *Salix capensis*, *Schotia latifolia*, *Juniperus oxycedrus*, *Jatropha elliptica*, and *Chamaecyparis lawsoniana* (Masika and Afolayan 2002; Karaman *et al.* 2003; Marquez *et al*. 2005; Smith *et al*. 2007). The stronger extraction capacity of methanol could have produced a greater number of active constituents responsible for antimicrobial activity. The results were more promising against the Gram-positive bacteria *S. aureus*. This could be attributed to the fact that the cell wall in Gram-positive bacteria has a single layer whereas the Gram-negative cell wall is a multi-layered structure (Yao *et al*. 1995; Ozcelik 1998), acting as a barrier to many environmental substances, including antibiotics (Tortora *et al.* 2001).

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