African Journal of Microbiology Research. Vol.(2) pp. 138-140, June, 2008
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2008 Academic Journals

Full Length Research Paper

Antibacterial activity of *Asperugo procumbens* L. against some human pathogenic bacteria

M. Ahanjan[1, 2]*, D. C. Mohana[1] and K. A. Raveesha[1]

[1]Microbiology Laboratory, Department of Studies in Botany and Microbiology, University of Mysore, Manasagangotri, Mysore 570 006, India.
[2]Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, I.R of Iran.

Accepted 23 May, 2008

Petroleum ether, chloroform ($CHCl_3$), ethanol (EtOH), methanol (MeOH) and aqueous (H_2O) extracts of leaves of *Asperugo procumbens* L. were evaluated for antibacterial activity against five human pathogenic bacterial strains with the agar-well diffusion method. The methanol extract was highly active against all the test bacteria, followed by the ethanol and aqueous extracts. The chloroform and petroleum ether extracts did not show any antibacterial activity. The minimum inhibitory concentration (MIC) of the methanol extract ranged between 1.56 and 12.5 mg/ml. The results of this study provide support for the use of *A. procumbens* L. in Iran for traditional medicines.

Key words: *Asperugo procumbens*, antibacterial activity, methanol extract, traditional medicine

INTRODUCTION

Plants have long formed the basis of sophisticated traditional medicine systems and natural products purportedly provide excellent leads for new drug development (Newman et al., 2000). Approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care and plants also play an important role in the health care system (Cragg et al., 1999). The rediscovery of the connection between plants and health is responsible for the launching of a new generation of multicomponent botanical drugs, dietary supplements, functional foods and plant-produced recombinant proteins (Raskin et al., 2002). Moreover, the emergence of multi-drug resistant (MDR) bacteria is of great concern to both clinicians and the pharmaceutical industry, since it is a major cause of treatment failure in many infectious diseases (Davies, 1994; Martino et al., 2002). Thus, it is necessary to search for alternative antimicrobial agents. One of the possible strategies towards this objective involves the identification and characterization of bioactive phytochemicals, which have antibacterial activity (Newman et al., 2000; Gottlieb et al., 2002). However, higher plants species in Iran have not been extensively surveyed for antibacterial activity (Bagheri and Regan, 1994).

*Corresponding author. E-mail: ahanjan2006@yahoo.co.in.

With the above in mind, the leaves of *Asperugo procumbens* L. were tested for antibacterial activity, since it is being used in traditional medicine. *A. procumbens* L. (Boraginaceae), more commonly known as German-madwort, is a herb with a slender stem that can grow up to 90 cm in length. The leaves of this plant are being used in Iran as a traditional medicine for treatment of skin infections, to strengthen the nervous system, as well as an antispasmodic and tranquillizer (Bagheri and Regan, 1994; Rechinger, 1999). There are, however, no published reports regarding the antibacterial properties of the plant. The aims of this study were therefore to prepare aqueous and solvent extracts from the leaves of *A. procumbens* and to screen the extracts quantitatively for antibacterial activity against five human pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Apparently healthy leaves of *Asperugo procumbens* L. were collected from the mountainous region of the Mazandaran province in Iran. A voucher specimen was deposited in the herbarium of the Department of Studies in Botany and Microbiology, University of Mysore, Mysore, India. Air-dried leaves and powdered samples were also hermetically sealed in separate polythene bags until the time of extraction.

Table 1. Antibacterial activity of leaf extracts of A. *procumbens*.

Bacteria	Aqueous extract	Petroleum ether extract	Chloroform extract	Methanol extract	Ethanol extract
E. coli	7±0.3	0.00	0.00	13±0.3	11±0.5
K. pneumoniae	9 ±0.5	0.00	0.00	25 ±0.4	17±0.5
P. aeruginosa	6 ±0.4	0.00	0.00	13 ±0.6	11±0.4
S. typhi	13±0.4.	0.00	0.00	18 ±0.5	17±0.4
S. aureus	8 ±0.5	0.00	0.00	17.5±0.5	15±0.3

Values presented are the mean ± SD of three replicates

Bacterial strains

Cultures of *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhi* (MTCC 733) and *Staphylococcus aureus* (MTCC 737) were obtained from the Microbial Type Culture Collection and Gene bank (MTCC), Chandigrah, India. The bacterial cultures were maintained on Mueller-Hinton agar (MHA)

Preparation of *A. procumbens* leave extracts

The leaves were washed thoroughly three times with running water and once with distilled water. The specimens were air-dried under shade and powdered. To prepare aqueous extracts, 20 g of thoroughly dried leaves was macerated in 200 ml sterile water with a Waring blender for 10 min, then filtered through a double-layered muslin cloth and the filtrate was subsequently centrifuged at 1 000 x *g* for 10 min. The supernatant was recovered and filtered through Whatman No.1 filter paper to obtain the aqueous extract. Solvent extracts were prepared by making use of a Soxlet extractor. For this purpose, 20 g of the shade-dried powdered leaves of *A. procumbens* was placed in the thimble and extracted successively with 200 ml of petroleum ether, chloroform, methanol and ethanol for 72 h or until a colorless extract was obtained. Each of the solvent extracts was concentrated separately under reduced pres-sure (Satish et al*.,* 1999; Harborne, 1998). After complete solvent evaporation, each of these solvent extracts was weighed and subjected to antibacterial activity assays. All of the prepared extracts were preserved aseptically in a brown bottle at 5ºC until further use.

Antibacterial activity assay

The antibacterial activity of the aqueous and solvent plant extracts were determined by the agar well-diffusion method on Mueller-Hinton Agar (MHA) medium. Using a cork borer, five wells (5 mm in diameter) were made in the agar medium (one in the center and four wells were at the corner) and inoculums containing 10^6 CFU/ml of the test bacteria were spread plated onto the surface of the medium with a sterile swab(Anon, 1985). In the case of aqueous extracts, 50 µl of the extract was pipetted into the wells, whilst 50 µl of sterilized distilled water served as a control. In the case of solvent extracts, 1.0 g of each of the concentrated solvent extracts (petroleum ether, chloroform, methanol and ethanol) was dissolved in 9.0 ml methanol and 50 µl of each solvent extract was pipetted into the wells, as described above, except that methanol served as control. The agar plates were incubated for at 37ºC for 24 h and the diameter of the zone of inhibition surrounding the wells was measured. Assays were performed in triplicate and the data are shown as the mean ± standard deviation (SD).

Determination of the minimum inhibitory concentration (MIC)

The MIC of the methanol extract only was determined, since it showed the highest antibacterial activity against all five test bacteria. A two-fold serial dilution of the extract was prepared in sterile distilled water to obtain a concentration range between 0.781 to 50 mg/ml. The MIC was determined as described above, except that a 100 µl volume of each dilution was used and the inoculum of the test bacteria was standardized to 5×10^5 CFU/ml. The agar plates were incubated at 37ºC for 24 h. The lowest concentration of extract showing a clear zone of inhibition was taken as the MIC. The MIC for each of the test bacteria was determined in triplicate assays and the data are shown as the mean ± SD.

Phytochemical analysis

Phytochemical analysis of all the evaporated solvent extracts was conducted according to the procedures of Anon (1985) and Harborne (1998).

RESULTS AND DISCUSSION

The results regarding the antibacterial activity of the aqueous and solvent extracts prepared from leaves of *A. procumbens* are indicated in Table 1. Whereas the methanol and ethanol extracts showed good activity against all the test bacteria, the aqueous extract displayed weaker activity, while the chloroform and petroleum ether extracts did not show any antibacterial activity. Of the different extracts, the methanol extract displayed the highest antibacterial activity, as was evidenced by it displaying the highest mean zone of inhibition against all of the test bacteria. The MIC value was 1.56 mg/ml for *K. pneumoniae, S. aureus* and *S. typhi*, and 12.5 mg/ml for *E. coli* and *P. aeruginosa* (Table 2). Phytochemical analysis indicated the presence, amongst other, of tannins, flavonoids and phenolics in the methanol extract. However, alkaloids and saponins were absent in the extract (Table 3).

A review of the literature revealed that information on the antibacterial potential of *A. procumbens* is lacking. Aqueous and different solvent extracts of leaves of *A. procumbens* were thus evaluated for their antibacterial potential, and the results of this study revealed highly significant antibacterial activity against the entire test bacteria in the methanol extract. None of the earlier reports

Table 2. MIC of methanol extracts of A. *procumbens* against the test bacteria.

	Methanol extract at different concentration (mg/ml)						
Bacteria	50	25	12.5	6.25	3.12	1.56	0.78
E. coli	13 ±0.3	5±0.5	0.00	0.00	0.00	0.00	0.00
K. pneumoniae	25 ±0.4	21±0.5	16±0.6	9±0.3	5±0.3	0.00	0.00
P. aeruginosa	13 ±0.6	6±0.3	0.00	0.00	0.00	0.00	0.00
S. typhi	18 ±0.5	15±0.3	12±0.4	7±0.4	4±0.3	0.00	0.00
S. aureus	18.5±0.5	15±0.5	12±0.4	7±0.3	4±0.3	0.00	0.00

Values presented are the mean ± SD of three replicates.

Table 3. Phytochemical analysis of the methanol extract of A. *procumbens* leaves.

Constituents	Methanol extract
Alkaloids	-
Carbohydrates	+
Flavonoids	+
Phenolics	+
Proteins	+
Saponins	-
Tannins	+

+, Present; -, Absent

have demonstrated or reported the antibacteria l potential of *A. procumbens* (Bagheri and Regan, 1994; Bonjar, 2004). This report therefore is the first to demonstrate the antibacterial activity of this plant. Preliminary phyto-chemical analysis of the methanol extract of leaves of *A. procumbens* revealed the presence of tannins, phe-nolics and flavonoids. Notably, both tannin and phenolics have been reported to possess antibacterial activity (Leven et al., 1979; Javanmardi et al*.,* 2002). Based on the limited spectrum of activity of the aqueous extract compared with the methanol extract (Table 1), it suggests that the active principle is more soluble in methanol than in the aqueous system. Thus, it is recommended that methanol be used as solvent for the large-scale extrac-tion of the active principle. Further investigations are in progress to isolate and characterize the antibacterial principle. In conclusion, this study provides support for the use of this plant in Iranian traditional medicine (Bagheri, 1994; Rechinger, 1999).

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