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Antimicrobial Screening of Medicinal Plant – *Artemisia pallens*

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Abstract: *Artemisia pallens* Wall, a medicinally important plant, belongs to family Asteraceae. It is This plant is used in Ayurvedic system of medicines. In order to search for antimicrobial activity of secondary metabolites, screening of aerial parts of *A. pallens* was carried out. Air shade dried powdered plant material was extracted using solvents of increasing polarity from non polar (n-hexane) , semi-polar (chloroform) to polar (methanol). Extracts were analyzed for their antibacterial capacity against six bacterial strains and an yeast strain. The antibacterial activity was determined by using disc diffusion method. *Bacillus cereus* was found to be more susceptible strain. Only methanol extract of *Artemisia pallens* showed the activity. Therefore, this was selected for further investigation to determine its therapeutic potential.
Key Words : Antimicrobial, *Artemisia pallens* , *Bacillus cereus*, Methanol extract.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (1). Over 50% of all modern clinical drugs are of natural product origin (2) and natural products play an important role in drug development programs in the pharmaceutical industries (3).

A. Pallens is a small and aromatic herbaceous plant which is native to the southern part of India, especially to the states of Karnataka, Tamil Nadu , Andhra Pradesh and in Maharashtra. In the regional languages of the south, it is known by several names as "davanam" in Tamil, "davanamu" in Telugu and "davana" in Kannada. Its leaves and flowers are highly valued in the making of floral decorations and oils. Leaves are very small, bluish green with yellow flowers and inconspicuous. It is utilized in traditional Ayurvedic medicinal formulations. Oral administration of the methanol extract of the aerial parts of Artemisia pallens Wall (Used in Indian folk medicine for the treatment of diabetes mellitus) led to significant blood glucose lowering effect in glucose-fed hyperglycemic and alloxan-induced diabetic rats. (4). Essential oil of davana is useful as antiseptic and disinfectant (5). The present work is carried out in order to evaluate the efficacy of the plant for its antimicrobial activity.

Experimental

Artemisia pallens was collected from Jejuri , Maharashtra state, India. It was authenticated at Botanical Survey of India, Pune. Its authentication number is BSI/WC/Tech/2008/1059.

Air shade dried and powdered plant material (10 g) was extracted with solvents (50 ml) of increasing polarity from non polar (n-hexane), semi-polar (chloroform) and the polar (methanol) by keeping for 24 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude extracts. Bioguided broad fractionation of methanol extract was carried to get four major fractions as, chloroform(A) ; chloroform:methanol 9:1 (B) ; chloroform:methanol 8:2 (C) and methanol.

Antimicrobial studies were carried out against six bacterial strains (*Escherichia coli* ATCC – 11246; *Staphylococcus aureus* ATCC – 6538 P; *Salmonella*

typhimurium ATCC – 23564; *Pseudomonas aeruginosa* ATCC – 27853; *Proteus vulgaris* ATCC – 13315; *Bacillus cereus* ATCC – 11778) and an yeast strain (*Saccharomyces cerevisiae* ATCC – 9763).

The paper disc diffusion method was employed. Samples of each extracts (200 mg) were dissolved in respective solvents (1 ml). Sterile 5 mm diameter filter paper discs were impregnated with 40 µL of these solvent extracts (8 mg /disc). The bacterial strains were inoculated on nutrient broth and incubated for 24 hours at 37 ± 0.1 °C while yeast strain was inoculated on nutrient broth and incubated for 48 hours at 25 ± 0.1 °C.

Adequate amount of Muller Hinton Agar and Chloramphenicol Yeast Glucose Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions. The count of the bacterial strains and yeast strain was adjusted to yield 1 X 10^7 to 1 X 10^8 mL^{-1} and 1 X 10^5 to 1 X 10^6 mL^{-1} respectively. The test organisms (0.1 ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with test organism were incubated for one hour before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with different extracts were placed on agar plates. The bacterial plates were incubated at 37 ± 0.1 °C for 24 hours while the yeast plates were incubated at 25 ± 0.1 °C for 48 hours.

After incubation all the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions. Streptomycin discs (10 µg/disc) and fluconazole discs (50 µg/disc) were used as positive controls.

Results and Discussion

Even if the plant was screened by other investigators, it was included in this study because different methods, different solvent extracts and different strains were used in the assay. This is the first report showing inhibition of *Bacillus cereus* by the methanolic extract. Thus this can be a new source for the drugs against food-borne illnesses.

The antibacterial activity of methanolic extract of *Artemisia pallens* is given in **Table 1** Non-polar and semi polar extract does not show any activity against the test organisms. Only methanolic extract showed activity against *Bacillus cereus*. Antimicrobial activity of fractions of this extract against *Bacillus cereus* is represented in **Table 2.** Fraction (C) chloroform : methanol (8:2) is found to be more active. Therefore, further aim is to isolate the compound of therapeutic potential from this extract.

Table I : Antimicrobial activity of plant extracts.

Micro organism	Gram	Diameter of zone of inhibition (mm)[a]	
		Methanol extract	Standards
Escherichia coli	- ve	-	20
Salmonella typhimurium	- ve	-	20
Staphylococcus aureus	+ ve	-	20
Pseudomonas aeruginosa	- ve	-	13
Proteus vulgaris	- ve	-	22
Bacillus cereus	+ ve	10	40
Saccharomyces cerevisiae		-	25

[a] Zone of inhibition including the diameter of filter paper disc (5 mm)
- = no activity.

Table II : Antimicrobial activity of fractions of methanol extract.

Fractions	Diameter of zone of inhibition (mm)[a]
A	09 mm
B	10 mm
C	13 mm
D	07 mm

[a] Zone of inhibition including the diameter of filter paper disc (5 mm)

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