

Induced Dwarf Mutant in *Catharanthus roseus* with Enhanced Antibacterial Activity

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Evaluation of an ethyl methane sulphonate-induced dwarf mutant of *Catharanthus roseus* (L.) G. Don revealed that the mutant exhibited marked variation in morphometric parameters. The *in vitro* antibacterial activity of the aqueous and alcoholic leaf extracts of the mutant and control plants was investigated against medically important bacteria. The mutant leaf extracts showed enhanced antibacterial activity against all the tested bacteria except *Bacillus subtilis*.

Key words: Antibacterial activity, *catharanthus roseus*, leaf extracts, mutant

Catharanthus roseus (L.) G. Don (Periwinkle), a perennial tropical plant of family *Apocynaceae*, produces a high number of monoterpenoid indole alkaloids of which two dimeric alkaloids, vincristine and vinblastine are clinically useful oncolytic drugs^[1]. These two bis-indole alkaloids occur in trace amounts in leaves and are products of oxidative coupling of catharanthine and vindoline. Periwinkle is also mentioned in folk-lore remedies for treatment of diabetes^[2], malaria^[3], dysentery^[4], insect bite^[5], kidney disorder^[6], irregular menstrual cycle^[7], and skin infections^[4]. Earlier, Virmani *et al.*^[4] reported antimicrobial effect of its plant parts against *Vibrio cholerae* and *Mycobacterium pyrogenous*. Chopra *et al.*^[8] reported the same against spinach mosaic virus in their *in vitro* studies. The pharmacological and therapeutic value of this plant has promoted intensive research for the development of its improved cultivars. Among various improvement methods, mutation breeding is one of the promising approaches, for development of such improved ideochemovars^[9]. Hence mutagenesis in *Catharanthus roseus* was attempted to induce the useful mutations and a study was undertaken for evaluation of morphological parameters of a dwarf mutant and the antibacterial activity of the aqueous and ethanol leaf extracts of the mutant and control plants against five medically important bacterial strains.

For mutagenesis study, seeds of *Catharanthus roseus* var. Nirmal (CIMAP 0865) were procured from Central Institute of Medicinal and Aromatic Plant, Lucknow and its ethyl methane sulphonate (EMS)-treated seeds were grown to raise the M₁ generation. On screening for induced variability, a dwarf variant was also isolated which being true breeding, was established as dwarf mutant. The leaves of control and dwarf mutant plants of periwinkle were collected from the experimental field of Botany department of Lucknow University. The leaves were thoroughly washed with distilled water, cut into small pieces and dried in an oven at 35° for 5 days. The leaves were homogenized to a fine powder in a mechanical

grinder. Ten grams of leaf powder was extracted with 100 ml ethanol with agitation on a rotary shaker (100 rpm) over night, and filtered through muslin cloth. The crude aqueous extract was prepared by heating slowly 10 g leaf powder with 100 ml distilled water for 6 h and filtering the extract through a muslin cloth. Both the extracts were concentrated to one fifth of the total volume. The *in vitro* screening of antibacterial activity was done against the gram positive *Staphylococcus aureus*, *Staphylococcus citreus*, and *Bacillus subtilis* and gram negative *Escherichia coli* and *Pseudomonas aeruginosa* bacteria by determining the zone of inhibition using agar disc diffusion method^[10,11].

The dwarf variants were isolated from M₁ population after 1% EMS treatment and establishing as mutants being true breeding in subsequent generations. The dwarf mutant exhibited decreased plant height, internodal length, corolla tube length, leaf breadth, petal length and breadth than that of control plants imparting a distinct phenotype to plants. The leaf extracts of mutant and control, both aqueous and alcohol, were found to inhibit growth of bacterial colonies, and the effect was more prominent with the extracts of the mutant leaves. Moreover, the effect of alcohol extracts on bacterial growth was more prominent. It has been reported that polarity of antibacterial compounds is crucial for their activity^[12] and the extracts prepared using ethanol were more active against bacterial species. It is evident that mutant leaf extracts had better antibacterial potential

TABLE 1: ZONE OF INHIBITION PRODUCED BY THE LEAF EXTRACTS OF CONTROL AND MUTANT PLANT OF CATHARANTHUS ROSEUS

Bacterial Strains	Control leaf extracts		Mutant leaf extracts	
	Aqueous	Ethanol	Aqueous	Ethanol
<i>S. aureus</i>	2.7	3.8	3	3.9
<i>S. citreus</i>	6	8	7	9
<i>B. subtilis</i>	2.6	3.9	0	0
<i>E. coli</i>	9	12	10	17
<i>P. aeruginosa</i>	5	7	6	8

Zone of inhibition (mm), including the diameter of the filter paper disc (4 mm); mean value of three independent experiments.

against *S. aureus*, *S. citreus*, *E. coli* and *P. aeruginosa* bacteria while *B. subtilis* was not affected (Table 1). The variation in antibacterial activity between mutant and control plant leaves might be due to the genomic changes, induced by the mutagen consequently influencing the synthesis and level of bio-active compounds like vincristine, vinblastine, vindoline in tissue, which might be responsible for antibacterial property of periwinkle leaves as also reported earlier^[13].

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