

Antibacterial Activity of *Cissus quadrangularis* Linn

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The *in vitro* antibacterial activity of different extracts of *Cissus quadrangularis* Linn (Vitaceae) against some Gram-negative and Gram-positive bacteria, were investigated. The methanol and ethyl acetate extract showed high activity against the bacteria tested.

Cissus quadrangularis Linn (Family: Vitaceae) is a perennial climber, found mostly in the hotter parts of the world such as India, Sri Lanka, Tropical Africa, South Africa, Thailand, Java, and Philippines¹. The plant is mentioned in the ancient systems of medicine such as Ayurveda, and is useful for treatment of bloody diarrhoea, skin disorders, earache², haemorrhoids, irregular menstruation, and accelerates healing of bone fracture³.

Murthy *et al.*⁴ have previously reported the antimicrobial

effects of the plant, along with the values of zones of inhibition. Here, we report the minimum inhibitory concentration (MIC) values of the different extracts of the plant stem using the microtitre plate method^{5,6}, which is relatively simple, reproducible, and rapid.

The stems of *Cissus quadrangularis*, collected from the plants maintained in the greenhouse were washed with water, cleaned, cut into small sections, and dried in an oven at 40° for 10-12 d. The dried material was ground to a fine powder in a homogeniser. The powder was stored in a refrigerator.

Extraction was carried out using a Soxhlet apparatus.

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Ethyl acetate, acetone, petroleum ether (40-60°), methanol (HPLC grade, Sisco Research Laboratories, Mumbai), ethanol, and water were the solvents used for extraction. The extracts were evaporated to obtain dry residues, and then solutions of known concentrations were prepared in respective solvents.

Bacillus subtilis (ATCC No. 6633), *Escherichia coli* (ATCC No. 25922), *Pseudomonas aeruginosa* (ATCC No. 27853), and *Staphylococcus aureus* (ATCC No. 25923), were obtained from National Centre for Industrial Microorganisms, National Chemical Laboratory, Pune. Clinical samples of *Salmonella typhi* and *Streptococcus pyogenes* were obtained from the cultures maintained in the department.

The bioassays were performed in 96-well sterile microtitre plates (Nunclon, Denmark). All the 96 wells of the plate were filled with 50 µl of sterile nutrient broth (HiMedia Laboratories). A 50 µl aliquot of the extract was then added to row A, and a 50% serial dilution was achieved (row A to H) by transferring 50 µl at a time to subsequent wells. In all, there were four controls; sterility control: 50 µl nutrient broth only, growth control: 50 µl nutrient broth + 50 µl culture, ampicillin control: 50 µl nutrient broth + 50 µl culture + 250 µg/ml ampicillin, solvent control: 50 µl nutrient broth + 50 µl culture + 50 µl solvent.

An aliquot of a 3 h culture (50 µl) of the test organism in nutrient broth was added in each well, except in the sterility control well. The covered microplates were incubated for 24 h at 37°. Bacterial growth was detected by adding 50 µl of 0.2 mg/ml solution of 2[4-iodophenyl]-3[4-nitrophenyl]-5-phenyl-2H-tetrazolium chloride (INT, from SRL) in each test well, and the plate was incubated further for at least 30 min at 37°, to ensure adequate colour development. The lowest concentration in which there was a definite decrease in colour was taken as the MIC of that extract, for that particular organism. All the experiments were performed in triplicates. The results obtained, were subjected to ANOVA and critical

difference analysis (CD analysis), at 5% level of significance.

The MIC values of different extracts against all the organisms tested, are detailed in Table 1. The ethyl acetate, acetone, and methanol extracts, showed antimicrobial properties and that *B. subtilis*, *P. aeruginosa*, *S. typhi*, *S. aureus*, and *S. pyogenes*, were susceptible to at least two extracts. Petroleum ether, ethanol, and water extracts, failed to inhibit the bacterial growth of the strains tested. *E. coli* did not respond to any of the extracts used.

ANOVA was carried out to test the significance of difference between the antimicrobial properties of the extracts (ethyl acetate, acetone and methanol), and difference between the susceptibility of microorganisms (*P. aeruginosa*, *S. typhi*, *S. aureus*). The analysis showed that there is a significant difference between the extracts (F value=8.84; F critical=3.42) and the microorganisms (F value=15.63; F critical=3.42).

Further, CD analysis was carried out to pinpoint the best extract, and the most susceptible microorganism. The analysis revealed that the methanol extract is significantly better than all the other extracts used (difference of means=2.04 and CD value=1.80), and *Staphylococcus aureus* is significantly more susceptible as compared to the other tested strains (difference of means=2.77 and CD value=1.80). All the tests were carried out, and the decisions were taken at 5% level. Further studies are needed to isolate and characterize the compound(s) responsible for the inhibitory activity.

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TABLE 1: MIC VALUES OF THE TEST ORGANISMS AGAINST VARIOUS EXTRACTS

Extract	MIC values in mg/ml					
	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
Petroleum ether	-	-	-	-	-	-
Ethyl acetate	-	0.93	1.87	3.75	0.93	3.75
Acetone	-	-	3.125	6.25	1.56	-
Methanol	-	0.465	3.12	1.24	0.465	0.93
Ethanol	-	-	-	-	-	-
Water	-	-	-	-	-	-

All values are expressed as mean of three experiments. '-' indicates no inhibition.

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