

In vitro evaluation of antibacterial activity of *Anona squamosa* and *Tribulus terrestris* leaf extracts

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SUMMARY

The effect of ethanol and aqueous extracts of leaves of *Anona squamosa* and *Tribulus terrestris* on the growth of Gram negative *Pseudomonas aeruginosa*, *Escherichia coli* and Gram positive *Staphylococcus epidermidis*, *Streptococcus agalactiae* bacterial strains was evaluated *in vitro* by Agar well diffusion assay method. The extracts inhibited the growth of all test organisms. Extract of *A.squamosa* exhibited greater antibacterial activity than *T.terrestris*. Gram positive strains were found more sensitive than Gram negative strains. Ethanol extract showed comparatively more inhibitory effect than aqueous extract. *Staphylococcus epidermidis* was found more sensitive to the leaf extracts of *A.squamosa* while *Streptococcus agalactiae* to that of *T.terrestris*. Zone of inhibition increased with increase in concentration of the extract. The results may be of importance in identification of new potential antibacterial compounds in plants.

Key words : *Anona squamosa*, *Tribulus terrestris*, Leaf extracts, Bacterial strains, Zone of inhibition

Plants are rich in wide variety of secondary metabolites such as tannins, terpenoides, alkaloids, flavonoides, phenols, essential oils which have antibacterial properties (Trease and Evans, 1972, Evans *et al.*, 1986, Cowan, 1999). Many natural antimicrobial compounds can be derived from plants (Gordon and David, 2001). Plants based antimicrobials have enormous therapeutic potential and may become the base for the development of new medicines. (Trease and Evans, 1972).

In recent years several workers (Ikram and Inamul, 1984, Naqvi *et al.*, 1991, Samy *et al.*, 1998, Dorman and Deans, 2000, Samy and Ignacimuthu, 2000, Srinivasan *et al.*, 2001, Kapoor *et al.*, 2007, Nair and Chanda, 2007 a and b, Seema *et al.*, 2007 and Sengottuvel *et al.*, 2007) screened many plants for antibacterial properties.

In this study, leaf extract of two plants, *A. squamosa* and *T. terrestris* were evaluated for potential antibacterial activity against the clinically significant two Gram negative bacterial strains, *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, and two Gram positive bacterial strains, *Staphylococcus epidermidis* ATCC12228 and *Streptococcus agalactiae* NCIM2401.

Anona squamosa belongs to Anonaceae family. It

is cultivated mainly for the edible fruits. All parts of plant are used in natural medicine in the tropics. It is considered to be good source of natural antibacterial compounds. (Oliver-Breuer, 1986). *Tribulus terrestris* is a naturally grown wild plant native to tropical and sub tropical regions of the world. It is classified under the family Zygophyllaceae. Different parts of *T. terrestris* is reported to possess antibacterial properties (Firas *et al.*, 2008).

MATERIALS AND METHODS

Fresh leaves of *Anona squamosa* and *Tribulus terrestris* were collected and washed in tap water. These leaves were then surface sterilised with 0.1% HgCl₂ for 1-2 minutes and again washed twice in sterile distilled water to ensure that the traces of HgCl₂ are removed and kept for drying in shade. The dried leaves were then ground into fine powder. 10 g of the powder was extracted in 100 ml ethanol and distilled water using soxhlet apparatus. Then the extracts were kept at room temperature for complete evaporation of solvent and water. The residue was mixed in appropriate amount of DMSO (Dimethyl Sulphoxide) to get the stock solutions of different concentrations *viz.*, 100, 200, 300, 400 and 500µg/µl and stored in glass tubes at 4°C for further use in determining the effect on the growth of Gram negative *Pseudomonas aeruginosa* and *Escherichia coli* and Gram positive *Staphylococcus epidermidis*, *Streptococcus agalactiae* bacterial strains

Gram negative and Gram positive bacterial strains procured from National Chemical Laboratory (NCL) Pune were maintained on Nutrient agar slants for use as test organisms.

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A loopful of 24 hrs old test organisms was added in 30 ml sterile nutrient broth for activation and shaken thoroughly to obtain uniform suspension.

The antibacterial activity of the plant extract was determined following the agar well diffusion method (Perez *et al.*, 1990).

0.2ml of inoculum suspension containing 10^8 cells/ml was inoculated in the molten Mueller-Hinton agar medium and kept on a rotary shaker. After proper homogenization, the medium was poured in sterile Petri plates and allowed to solidify. Then with a sterile cork borer a 6 mm well was made in the centre. 0.1ml of different concentrations (100, 200, 300, 400 and 500 μ g/ μ l) of ethanol and aqueous plant extract was put in each well for bioassay. The test plates were incubated at 37°C. Positive as well as negative control sets with Tetracycline and double distilled water in the wells, respectively were kept for each bacterial strain. After 24 hours, the diameter of zone of inhibition was measured.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented in Table 1 and 2.

The ethanol and aqueous leaf extracts of *A. squamosa* and *T. terrestris* inhibited the growth of all the test organisms thus demonstrating antibacterial properties. However, the growth of Gram positive strains were found more inhibited than Gram negative strains. Nair and Chanda (2007b) also reported Gram positive strains more sensitive. Ethanol extract showed more effect than aqueous extract in inhibiting the growth of the bacterial strains which is in conformity with the investigations reported by Nair and Chanda (2007a) and Firas *et al.* (2008). Among Gram positive strains, *Staphylococcus epidermidis* was found more sensitive to the leaf extracts of *A. squamosa* while *Streptococcus agalactiae* to that of *T. terrestris*. Diameter of the zone of inhibition is directly proportional to the concentration of the extract. more

The drug resistant bacteria, *E. coli* and *P. aeruginosa*

Table 1 : Effect of ethanol and aqueous leaf extracts of *A. squamosa* on the growth of bacteria

Sr. No.	Extract concentration (μ g/ μ l)	Zone of Inhibition in (mm)							
		<i>Pseudomonas aeruginosa</i>		<i>Escherichia coli</i>		<i>Staphylococcus epidermidis</i>		<i>Streptococcus agalactiae</i>	
		A	B	A	B	A	B	A	B
1.	100	9	6	10	7	13	9	11	8
2.	200	10	7	12	8	14	11	13	10
3.	300	13	10	14	11	16	13	15	12
4.	400	16	14	15	13	19	16	18	15
5.	500	18	15	17	15	21	18	20	17
6.	+ve Ctrl:-Tetracycline (500 μ g/ μ l)		12		11		17		16
	-ve Ctrl: Double distilled water		Nil		Nil		Nil		Nil

A = Solvent extract

B=Aqueous extract

Table 2 : Effect of ethanol and aqueous leaf extracts of *T. terrestris* on the growth of bacteria

Sr. No.	Extract concentration (μ g/ μ l)	Zone of Inhibition in (mm)							
		<i>Pseudomonas aeruginosa</i>		<i>Escherichia coli</i>		<i>Staphylococcus epidermidis</i>		<i>Streptococcus agalactiae</i>	
		A	B	A	B	A	B	A	B
1.	100	7	6	8	6	9	7	9	8
2.	200	8	6	8	7	10	7	11	9
3.	300	10	7	9	7	11	8	12	10
4.	400	12	9	11	8	14	9	13	12
5.	500	13	9	12	9	15	11	17	13
6.	+ve Ctrl : Tetracycline (500 μ g/ μ l)		12		11		17		16
	-ve Ctrl: Double distilled water		Nil		Nil		Nil		Nil

A = Solvent extract

B= Aqueous extract

were also inhibited by the extracts. But Gislene *et al.* (2000) reported that *E.coli* did not show any sensitivity to the plant extracts. Among Gram positive strains, *S. epidermidis* showed maximum zone of inhibition.

Conclusion:

Investigations indicate that the higher plants could be a rich and alternative source for obtaining great number

of antimicrobial compounds with an enormous therapeutic potential. Continuous and further exploration of plant antimicrobials is thus needed.

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