Short comunication

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Antibacterial efficacy of methanolic extracts of *Allamanda. cathartica* Linn. and extract of *M. aeruginosa*

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Abstracts

Secondary plant metabolites shows a wide range of biological activity. Plant products are used as nutraceuticals. antioxidants, anticancerous ant antimicrobial agents. Extracts of M. aerugenosa and A. cathartica Linn. s*howed remarkable antibacterial activity against human pathogenic bacteria. Antibacterial activity of extracts were determined by filter paper disc method.*

Key words – Antibacterial, Inhibition zone

Introduction

The higher plants have been used in traditional medicine since long for cure of human diseases, because of their long history of use plant products are believed to be safer than synthetic drugs. India ranks for most after South Korea in the supply of medicinal plants to industrialized country where demands for natural drugs have been increasing in recent years (Chadha *et.al., 1*980). Besides plants microbes are the major source of bioactive antimicrobial agents. In present study alcoholic extracts of *M. aeruginosa and Allamanda cathartica* Linn. were screened for antibacterial activity against some human pathogenic bacteria. During recent years interesting work is being carried out to screen some important medicinal plants and to ascertain their bioactive principles with the help of modern techniques (Tiwari and Dubey, 1996).

Materials and methods

Toxin extraction and testing

The toxin was extracted by the method described by Siegelman *et al.,* (1984). In brief lyophilized cells were stirred in 20-200 ml of 5% n-butanol, 20% methanol (v/v) in water for 1-2 h at 4 ^{0}C. The suspension was filtered through Whatman filter disc and alcohol content of filtrate was evaporated at 40 ^{0}C in rotary vacuum evaporator. The samples were eluted from C-18 bond-pack columns and elute was finally separated by HP LC (Waters) using a reverse phase column in acetonitrileammonium acetate solvent at

the flow rate of 1.5 ml/min at 240 nm. The peaks published by Ohtake et al. (1989), were used as reference standard. The organic extractions were made using methanol and ethylacetate in acidic, neutral and alkaline conditions. The most pronounced antimicrobial effects were obtained with methanol-acetic acid extracts and this method of extraction was used in the studies with the strains of *M. aeruginosa*. After removing the supernatant fluid from the water extraction, 3ml of methanol and 100 µ1 acetic acid were added. The sample was mixed and shaken for 10 seconds and was then left with the extraction fluid for 10 min. The sample was centrifuged at 2500 rpm for 6 min and the supernatant fluid was then transferred to a cleared glass-stoppered tube. The organic solvent was evaporated to dryness under a stream of N_2 gas at 40^0C and a Rotary evaporating unit (Remi, India). The dry residue was resuspended in 1 ml methanol the final concentration was equivalent to 50 mg of freeze-dried cyanobacteria. Fresh leaves of *Allamanda cathartica* Linn were collected from Botanical Garden of Banaras Hindu University, Varanasi, India. A specimen sample is kept in the department. Air dried leaves (500 g) were extracted with methyl alcohol in a soxhlet apparatus. The Solvent was removed by distillation in vacuum. The resultant gummy mass (107gm) was dried in vacuum desiccators over anhydrous calcium chloride. The methyl alcohol extracts were chromatographed over silica gel with solvent of increasing polarity. Extract obtained in benzene and methanol was screened for antibacterial activity. To test the toxicity of water or organic extracts, sterile filter discs with a diameter of 12.7 mm (Millipore) were soaked in the extract solutions obtained in previous experiment and kept overnight at room temperature to evaporate the methanol. Control filter discs were soaked in sterilized distilled water or 75 µl methanol and evaporated.

Screening of extracts of *A. cathartica* and extract of *M. aeruginosa*

Culture were lawned on nutrient agar medium in Petri-plate and filter discs impregnated with methanol extracts of different concentration of extracts were stored in petridishes at room temperature for 24 hour and the inhibition zones were measured (Dahia and Thind, 1976).

Results and Discussion

There is remarkable inhibition zone obtained around filter paper against different human pathogenic bacteria. This result indicates that alcoholic extracts of *M. aerugenosa* and *A. cathartica.* shows antibacterial activity (Table-1). Pure Crystals of these extracts may be isolated and their chemical structure can be elucidated by spectroscopic studies. These characterized chemicals can be further studied for detailed biological activity.

Table-1 Effecc of extracts of *M. aeruginosa* and *A. cathartica* against human pathogenic bacteria

Extracts	Inhibition zone in mm				
	E.coli	*Klebsiella. Sp.*	*Pseudomonas sp.*	*Staphylococcus sp.*	*V. cholorae* 569B
M.aerugenosa	16	18	17	18	15
A.cathartica.	15	20	21	20	16

Conclusion

Extracts of *M. aeruginosa* and *A. cathartica* shows remarkable antibacterial activity against human pathogenic bacteria viz. *E. coli, Klebsiella sp. Pseudomonas sp, Staphylococcus sp* and *Vibrio cholorae* . Compound responsible for antibacterial activity in these extracts can be isolated by chromatographic methods and their chemical structure can be elucidated. The pure and identified compound can be further studied for their effect on different mammalian system. The compound which is found non toxic against mammalian system may be standardize as antibacterial agent for the cure of different diseases caused by the above mentioned bacteria.

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