Antimicrobial Activity Of Thevetia Peruviana (Pers.) K. Schum. And Nerium Indicum Linn.

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Citation

Abstract
The main objective of the present study was to analyze the antimicrobial potential of various concentrations (2mg, 4mg, 6mg and 8mg/well) of leaf extracts of Thevetia peruviana (Pers.) K. Schum. and its close relative Nerium indicum Linn. (Apocynaceae). The dried leaves of these plants were extracted with 95% alcohol using soxhlet apparatus. The obtained extracts were concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature, (40 - 50°C) and note down the yield of the crude extracts, then subjected to antimicrobial activity for the assessment of inhibitory effects of these plants against ten medically important antibiotic resistant pathogenic microbes by in vitro agar well diffusion method. The results of the present study clearly demonstrated that, the extracts of both the plants significantly inhibited the growth of all the pathogens used in this study in a dose dependent manner. The extract of Thevetia peruviana proved to be effective against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, where as, Proteus vulgaris showed susceptibility only at higher doses. Thevetia extract also showed moderate antibiotic activity against Staphylococcus aureus, Candida albicans, Aspergillus niger, Mucor, Rhizopus and Penicillium species. The alcoholic extract of Nerium indicum was effectively inhibited the growth rate of Staphylococcus aureus, Candida albicans, Aspergillus niger, Mucor, Rhizopus and Penicillium species even at lower concentrations. The same extract was also effective to other tested pathogens only at higher concentrations. In conclusion, the different concentrations of Thevetia peruviana and Nerium indicum have to possess non-specific broad spectrum antimicrobial activity.

INTRODUCTION
Medicinal plants use is widespread. The production of medicines and the pharmacological treatment of diseases began with the use of herbs. Plants historically have served as models in drug development for some major reasons. The first being that each plant is a unique chemical factory capable of synthesizing large number of highly complex and unusual chemical substances. The second reason involves biologically active substances derived from plants have screened as templates for synthesis of pharmaceuticals. While the reason concerns the fact that highly active secondary plant constituents have been instrumental as pharmacological tools to evaluate physiological processes. One of the paramount reasons for pursuing natural products chemistry resides in the actual or potential pharmacological activity to be found in alkaloids, terpenoids, coumarins, flavonoids, lignans etc.

Due to the diverse medicinal properties attributed to the plants, the present investigation was undertaken to analyze the antimicrobial potential of two locally available wild plants namely, Thevetia peruviana (Pers.) K. Schum. and Nerium indicum Linn. They are poisonous, ornamental, evergreen shrubs, belongs to the family Apocynaceae. The sap of these plants known to possess cardiac glycosides and are toxic to cardiac muscle and the autonomic nervous system (Eddleston et al., 2000; Langford and Boor, 1996; Pearn, 1989; Kurian, 2001)

MATERIALS AND METHODS
Collection of Plant materials: The leaves of Thevetia peruviana and Nerium indicum were collected from in and around Gulbarga city, Karnataka, India. All these plants were authenticated and the voucher specimens were deposited in Herbarium of Department of Botany, Gulbarga University, Gulbarga, India. Later leaves of these plants were subjected to surface sterilization using 50% alcohol and then shade dried for further analysis.

Preparation of the Extracts: The 100g leaves of D. stramonium and N. indicum were extracted with 95% alcohol at 60 - 80°C in a soxhlet apparatus. The different extracts were collected in a separate container and
concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of crude extracts.

Microorganisms Used: Clinical laboratory bacterial isolates of Klebsiella pneumoniae, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Candida albicans, Mucor, Rhizopus and Penicillium species were collected from the standard stock cultures of Microbiology Laboratory, Basaveshwara Hospital, Gulbarga, Karnataka, India. The bacterial and fungal cultures were maintained on nutrient agar and Sabouraud Dextrose Agar medium respectively, and were stored at 4°C for determining antimicrobial activity of some medicinal plants.

Culture Media: The nutrient agar, nutrient broth and Sabouraud dextrose agar were purchased from HiMedia, Laboratories Limited, Mumbai. Nystatin an antifungal agent, purchased from, Tocelo chemicals, Netherlands, Streptomycin (powdered form) another antibiotic from Nanjing Asian chemicals Co., Ltd. The solvents and other chemicals used were analytical grade.

Antimicrobial activity: Here the in vitro antibacterial and antifungal activities were assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar plate and Sabouraud dextrose agar plates for bacterial and fungal cultures. The wells were bored with 6mm borer in seeded agar and then the particular concentrations (2mg, 4mg, 6mg and 8mg/0.2ml/well) of the plant extracts and 10µg/0.2ml/well of streptomycin and Nystatin were added in each separate well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. Later, the zone of inhibition was measured and recorded (Yogesh Biradar et al., 2008; Uma Reddy et al., 2008).

Statistical Analysis: All the data are expressed as mean ± S.E.M. (standard error of the mean). The significance level was determined using the Student’s t test. A p-value of <0.05 was considered statistically significant.

RESULTS

It is evident from the results that, the extracts of both the plants significantly inhibited the growth of all the tested pathogens in a dose dependent manner. The test organisms like Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa were proved to be maximum level of susceptibility to Thevetia peruviana extract. Where as, same drug was effectively inhibited Proteus vulgaris only at higher doses, it has also showed moderate antibiotic activity against Staphylococcus aureus, Candida albicans, Aspergillus niger, Mucor, Rhizopus and Penicillium species. (Table-1). Similarly, Staphylococcus aureus, Candida albicans, Aspergillus niger, Mucor, Rhizopus and Penicillium species were found to be highly sensitive to Nerium indicum even at lower concentrations, where as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris and Penicillium species were found to be sensitive only at higher doses (Table-2). The obtained results were compared with standard control (Streptomycin as standard reference for antibacterial agent and Nystatin as standard antifungal agent) as well as negative control (0.2ml of double distilled water/ well).

DISCUSSION

The results imply that, the extracts of both the plants exhibited more or less pronounced antibacterial and antifungal potencies affecting Gram positive, Gram negative bacteria and fungi used in the present study. Several earlier reports also supporting the present research study. The chloroform and methanolic extracts of Nerium oleander roots bark and leaves showed high antimicrobial activity against Bacillus pumilus, Bacillus subtilis, Staphylococcus
aureus, Escherichia coli and Aspergillus niger (Hussain and Gorsi, 2004) The abilities of the alcoholic extract of Nerium oleander leaves acted as effective sources of wood preservative to suppress attack by Postia placenta (Fr.) (brown rot) and Trametes versicolor (L.: Fr.) Quel. (a white-rot) [Plant pathogenic fungi] was investigated (Osman Goktas et al., 20007). The ethanol extracts of Nerium floral parts were effectively inhibited the growth of several plant pathogenic fungi Fusarium oxysporum and Fusarium solani (Hadizadeh, 2009). Nerium indicum showed the highest antiinfluenza viral activity with 50% inhibitory dose of 10μg/ml (Rajbhandari et al, 2001). Alcoholic extract of Thevetia peruviana seeds was effective against the fungus Cladosporium cucumerinum (Llgia Gata et al., 2003). The alcoholic extract of Thevetia nerifolia showed antimicrobial activity in a range of 75-1200μg/ml (Rajesh Dabur et al, 2007).

CONCLUSION
In conclusion, the alcoholic extracts of Thevetia peruviana and Nerium indicum leaves could be an excellent source of antibiotic drugs against antibiotic resistant Gram positive, Gram negative bacteria and fungi used in this study. Probably this might be happened because the extracts of both the plants may contain active antibiotic principles with strong inhibitory effects against all test organisms. These plants represent novel leads and future studies may allow the development of a pharmacologically acceptable antimicrobial agent or class of agents.

References
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