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Original Research Article

ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF INCARVILLEA EMODI (ROYLE EX LINDL.) CHATTERJEE

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ABSTRACT

The present study has been designed to evaluate the antimicrobial and cytotoxic activities of crude aqueous and methanolic extracts of Incarvillea emodi along with its active polyamide column fractions. The in-vitro cytotoxic activity of two polyamide column fractions was performed by CVV assay method against CHOK 1 (Chinese hamster ovary cell line). The crude aqueous and methanolic extracts (1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml concentration) of I.emodi were taken for antimicrobial activity against four bacteria and three fungi. Effect of inhibition of cell growth by two polyamide column fractions showed significant cytotoxicity against CHO-K1 with PGI 94.1 % and 93.89 %. The results obtained from the study indicate comparatively good antifungal activity than antibacterial activity. The present study concluded that the aqueous and methanolic extracts and two polyamide column fractions of Incarvillea emodi possess potent antimicrobial and cytotoxic activities respectively. Further investigations are in progress to isolate pure compounds from the two active polyamide column fractions and their cytotoxic effects against CHO-K1 cell line.

Keywords: Incarvillea emodi, Aqueous extract, Methanol extract, Antimicrobial activity, Cytotoxic activity.

INTRODUCTION

Resistance of microbes is a natural biological phenomenon and continuous increase in this microbial resistance has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of HIV infections [1,2 ,3, 4 ,5] With the discovery of antibiotics in the 1950s, the plant derivatives usage as antimicrobials has been virtually absent. In the late 1990s, the use of plant extracts and other alternative forms of medical treatments is enjoying great popularity. The reason for this renaissance include, a reduction in the new antimicrobial drugs in the pharmaceutical pipeline, an increase in antimicrobial resistance and the need of treatments for new emerging pathogens [6]. Moreover, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of disease but also often with adulterations and side effects [7]. For the discovery of new lead compounds, scientists from different areas are struggling to investigate new plants for the detection of secondary plant constituents with their antimicrobial effects and synthesized further for their improved activity [8, 9, 10, 11]. The uncontrolled cell proliferation which is considered as serious diseases that threaten to human health is known as cancer. The causative agents of this dangerous disease are free radicals which are originated from cigarette smoking, air pollution, UV radiation, malnutrition and normal body functions. For these reasons, anticancer compounds are pulling the attention of researchers to isolate them from natural source for drug discovery [12].

The aim of this work was to carry out an antimicrobial screening of Incarvillea emodi crude aqueous and methanolic extracts of aerial parts (ABD-Ap-aq, ABD-Ap-M) and roots (ABD-Rt-Aq and ABD-Rt-M) collected from Abbottabad and aerial parts (K-Ap-Aq and K-Ap-M) collected from Muzaffarabad-AJK, along with its three polyamide column fractions. Also to check the cytotoxic effects of two active polyamide column fractions (PC. Fr. 19-27 and PC. Fr. 33-38) against CHO-K1 cell line. To the

best of our knowledge, there is no antimicrobial and cytotoxic activity studies on I. emodi aqueous and methanolic crude extracts.

MATERIALS AND METHODS

Plant collection

Plant was collected during April, 2012 in Himalayan region of District Abbottabad, Khyber Pakhtunkhwa Province and Muzaffarabad-AJK, Pakistan. Plant was identified by Dr. Uzma Khan, Assistant Professor, Department of Botany, Hazara University, Mansehra. Voucher specimens were deposited in the Central Herbarium of Hazara University.

Extract preparation

The plant material was dried in the shade and after drying the powdered materials were extracted with methanol. For aqueous extract of the plant, slurry was transferred to separating funnel. Removal of chlorophyll was done with the addition of petroleum ether. After complete drying at 40^0C by rotary evaporator, this aqueous extract was further processed by lyophilization and then the extract was stored for further analysis.

Antimicrobial activity

Antimicrobial activities including antibacterial and antifungal were determined as minimum inhibitory concentration (MIC) values using the broth micro dilution method following the procedures reported by the Clinical and Laboratory Standards Institute (CLSI) against the bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213) and the fungi (Candida albicans ATCC 90028, C. krusei ATCC 6258, C. parapsilosis ATCC 90018). The inoculum densities were approximately $5x10^5$ cfu/mL and $0.5\text{-}2.5x10^3$ cfu/mL for bacteria and fungi, respectively. Ciprofloxacin and fluconazole were used as reference compounds for antibacterial and antifungal activities respectively. MICs were determined by broth micro dilution method reported by the CLSI. Antibacterial activity test was performed in Mueller-Hinton Broth (Difco, USA). RPMI-1640 medium with L-glutamine (ICN-Flow, USA) buffered with 3-(N-morpholino) propanesulphonic acid (MOPS) (ICN-Flow, USA) was used as the culture medium for antifungal activity test. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity [13].

Determination of cell viability by CVV Assay

For cytotoxicity evaluation, 5 mg of each extract was taken into a labeled sterile tube. Samples were dissolved in 62.5µl of 100% ethanol then added 62.5 µl of water (concentration= 40 mg/ml in 50% ethanol). 2 ml of sterile DME was placed in a labeled sterile plastic tubes and added 20 µl of extract solution to it (concentration= 400µg/ml in DME with 5% ethanol). Following concentrations were used i.e. 1, 2, 5, 10, 20, 50, 100 and 200 µg/ml and all experiment was done in triplicates. Cultures were thawed for CHO-K1 cell line and passaged it in Dulbecco's modified Eagle's (DME) medium in 10% calf serum. Cells were suspended with trypsin/EDTA in 10 % calf serum in DME by washing the cultures with sterile medium, covering with sterile EDTA in Puck's saline/trypsin, drawing off the releasing solution, suspending the cells in 10 % calf serum in DME by vigorous pipetting. The cells were counted on a hemocytometer and the inoculum was diluted to add 100 µl of 2×10^4 cells/ml to each well. The wells were filled with formal saline, added gently by allowing it to flow into the wells. Trays were fixed for 30 minutes and then washed under tap water. Each well was stained with 0.5 % crystal violet in 20 % aqueous methanol by adding 2-3 drops. The trays were washed again under tap water to remove unbound stain and kept for drying in room temperature. 100 µl of DMSO was added to each well and rocked to mix. The absorbance was measured using a microplate reader at a wavelength of 562 nm. The percentage growth inhibition (PGI) was calculated using the following formula:

% Growth Inhibition = 100 – {(Mean OD of individual test group / Mean OD of control group) × 100} [14]

RESULTS AND DISCUSSION

According to [15], scientists have shown their interest in the isolation of bioactive antimicrobial compounds from medicinal plant which control microbes. Bioactive constituents and some food ingredients of vegetables and medicinal plants are significantly used as an antimicrobial agent [16]. For

the determination of antimicrobial susceptibility, broth dilution method is one of the most widely used methods. It is a simple procedure for testing small number of isolates, even a single isolates [17]
In the present study, antimicrobial potential of six crude extracts (including three aqueous and three methanolic) and three selected polyamide column fractions of Incarvillea emodi was screened against four bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213) and three pathogenic fungi (Candida albicans ATCC 90028, C. krusei ATCC 6258, C. parapsilosis ATCC 90018) using broth micro dilution assay. Total eleven concentrations i.e. 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml were tested against bacteria and fungi. Lowest concentration of the extract which inhibits any visual microbial growth was considered to be minimum inhibitory concentration (MIC). In general, all extracts of the plant exhibited antibacterial activity and their MICs were recorded in the range of 512-1024 µg/ml (Table 1). Plant extracts and fractions with MICs values < 1000 µg/ml were considered active in this study. In our investigation, ABD-Ap-Aq extract showed activity against P. aeruginosa, E. faecalis and E. coli (MICs= 512 µg/ml each) while remained inactive against S. aureus (MICs > 1000 µg/ml). ABD-Ap-M, ABD-Rt-Aq, ABD-Rt-M, K-Ap-Aq, and K-Ap-M exhibited no activity against S. aureus and P. aeruginosa (MICs > 1000 µg/ml) while active against E. faecalis and E. coli (MICs= 512 µg/ml). Polyamide column fractions also showed good activity against all the tested bacteria in this study. Fraction (PC. Fr. 19-27) showed activeness against S. aureus, P. aeruginosa and E. coli (MICs= 512 µg/ml) while inactive against E. faecalis (MICs > 1000 µg/ml). Second fraction (PC. Fr. 33-38) gave activity against P. aeruginosa, E. faecalis and E. coli (MICs= 512 µg/ml) but has no activity against S. aureus (MICs > 1000 µg/ml) while in contrast to this, PC. Fr. 44-46 showed the same activity but remained inactive against E. coli instead of S. aureus.
Table 2 summarizes the antifungal results of all the extracts of Incarvillea emodi. All extracts showed antifungal effect against the tested pathogenic fungi and MICs were recorded in the range of 32-256 µg/ml. Regarding the antifungal activity, all the plant extracts (aqueous and methanolic) and fractions screened, in general, were more active against fungi than bacteria. The most effective extracts of the plant against fungi were the three polyamide fractions. As shown in the Table 2, PC. Fr. 44-46 displayed the highest broad-spectrum antifungal activity among all the extracts of the plant tested (MICs= 32-128 µg/ml). For the other two fractions (PC. Fr. 19-27 and PC. Fr. 33-38) MICs were recorded in the range of 64-128 µg/ml against all the three fungi. Among the crude extracts of the plant, K-Ap-M showed strong antifungal activity against all the fungi used (MICs= 64-128 µg/ml). For the other crude extracts of the plant (ABD-Ap-Aq, ABD-Ap-M, ABD-Rt-Aq, ABD-Rt-M and K-Ap-Aq) MICs were recorded in the range of 128-256 µg/ml. In our continuous studies on CHO-K1 cell line, previously we have shown the comparative cytotoxic effects of three aqueous and methanolic crude extracts of I. emodi against CHO-K1 cell line (Manuscript accepted). Here in the present investigation, two polyamide column fractions (PC. Fr. 19-27) and (PC. Fr. 33-38) were also tested for their comparative cytotoxicity against CHO-K1 cell line. For both fractions, concentrations were used in the range of 1, 2, 5, 10, 20, 50, 100 and 200 µg/mL. At highest concentration 200 µg/mL, PC. Fr. 33-38 showed maximum inhibition (94.1 %) which is comparatively higher than the PC. Fr. 19-27 (Figure 1 and 2).

CONCLUSION

This is the first ever report on antimicrobial activity of aqueous and methanolic crude extracts of I. emodi along with the polyamide column fractions tested against CHO-K1 cell line. Results conclude that almost all samples showed moderate antibacterial activity against all the tested bacteria. Regarding the antifungal activity, all the plant extracts (aqueous and methanolic) and fractions screened, in general, were more active against fungi than bacteria. The most effective extracts of the plant against fungi were the three polyamide fractions. PC. Fr. 44-46 displayed the highest broad-spectrum antifungal activity among all the extracts of the plant tested.

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Table 1: MICs of plant extracts against bacteria by broth micro dilution assay

Plant extracts	*Staphylococcus*	*Pseudomonas*	*Enterococcus*	*Escherichia*

	aureus **ATCC 29213**	***aeruginosa*** **ATCC 27853**	***faecalis*** **ATCC 29212**	***coli*** **ATCC 25922**
ABD-Ap-Aq.	1024	512	512	512
ABD-Rt-Aq.	1024	1024	512	512
K-Ap-Aq.	1024	1024	512	512
ABD-Ap-M	1024	1024	512	512
ABD-Rt-M	1024	1024	512	512
K-Ap-M	1024	1024	512	512
PC.Fr.19-27	512	512	1024	512
PC.Fr.33-38	1024	512	512	512
PC.Fr.44-46	512	512	512	1024
Gentamicin	0.25	0.5	16	0.25

Table 2: MICs of plant extracts against pathogenic fungi by broth micro dilution assay

Plant extracts	***Candida albicans*** **ATCC 90028**	***Candida krusei*** **ATCC 6258**	***Candida parapsilosis*** **ATCC 90018**
ABD-Ap-Aq.	256	256	128
ABD-Rt-Aq.	128	256	128
K-Ap-Aq.	256	256	128
ABD-Ap-M	128	128	128
ABD-Rt-M	128	128	128
K-Ap-M	128	128	64
PC.Fr.19-27	128	128	128
PC.Fr.33-38	128	128	64
PC.Fr.44-46	128	64	32
Fluconazole	0.5	16	0.5

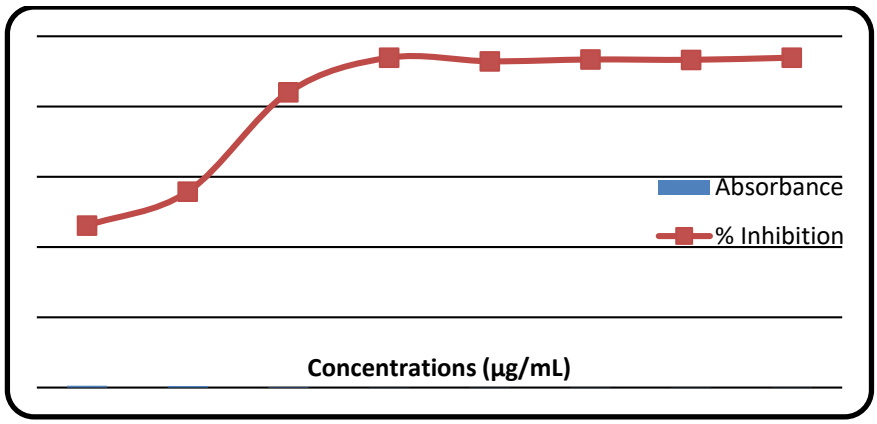

Figure 1: Effects of I. emodi PC. Fr. 19-27 against CHO-K1 cell line

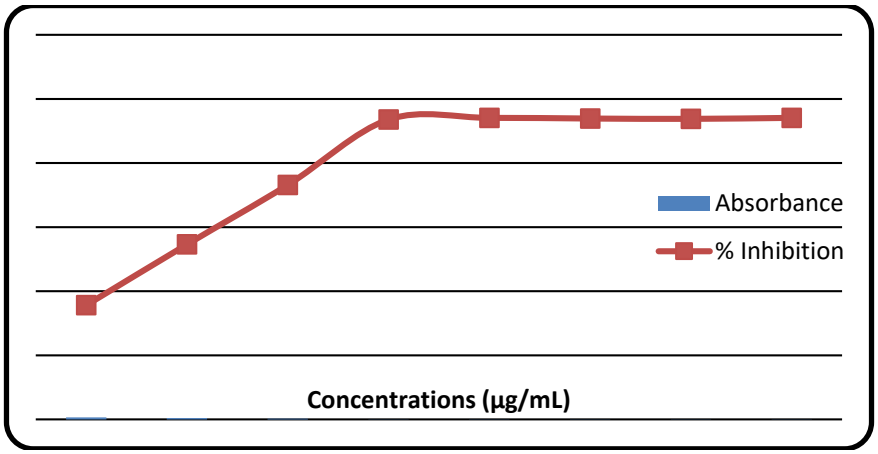

Figure 2: Effects of I. emodi PC. Fr. 33-38 against CHO-K1 cell line

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