Name of the Candidate: Sheikh Imran Ahmad

Name of the Supervisor: Dr. Fehmeeda Khatoon

Department: Applied Sciences & Humanities

Title: *In vitro* antimicrobial activity and phytochemical analysis of different plant extracts

Abstract

Introduction: The drug-resistant fungal and bacterial pathogens have complicated the treatment of infectious diseases in immune compromised, AIDS and cancer patients. Apart from resistance, some antibiotics have serious undesirable side effects which limit their applications, so there is urgent need to develop new antimicrobial substitutions that are very effective with minimal unwanted side effects, and higher plants represent a potential source of novel antibiotic prototypes. In this study we have focused to examine the antimicrobial potential of methanolic extracts of *C. cassia* bark (CCE) and *J. regia* root (JRE) by performing multiple antimicrobial assays. **Methods:** Phytochemical analysis of methanolic extracts of CCE and JRE have been carried through standard procedures. GC-MS analysis was used to determine the chemical composition of the extracts. In antimicrobial screening methods, MIC was determined *in vitro* in liquid medium by serial broth dilution method. In growth curve assays growth was recorded at pre-determined time periods at 595 nm, and compared with control. In spot assay yeast culture was spotted onto YNB plates in absence and presence of the test extracts and growth differences were recorded. For disc diffusion assay approximately 10^5 cells/ml were inoculated in molten agar media, and then sterile filter discs were impregnated with test extracts and placed on agar plates. In confocal microscopy propidium iodide were added to the cell suspensions to determine the general and nuclear morphology of the cell. In electron microscopy, test extracts equivalent to MIC concentration were added to the cells ($\sim 1\times10^6$) and incubated for 14 hours at 30ºC. The sample preparation and analysis methods were similar to those described by Shreaz et al 2013. Standard protocol as described by Mardegan et al 2006 was followed for proteinase and phospholipase secretion activity in absence and presence of the test extracts. **Results and Discussion:** Phytochemical results of CCE showed the presence of phenols, alkaloids, steroids, tannins and absence of saponins and glycosides, while as JRE showed the presence of phenols, alkaloids, steroids, saponins, tannins

and absence of glycosides. In GC-MS analysis, a total of 35 and 38 phytochemical compounds were identified from the CCE and JRE extracts. JRE showed the MIC ranging from 300-700 µg/ml and CCE showed the MIC ranging from 800-1600 µg/ml against different *Candida* isolates. In case of bacterial isolates the range of MIC for JRE was 1800-1900 µg/ml and for CCE it was in the range of 2100-2200 µg/ml. Antifungal and antibacterial activity on solid media examined by disc diffusion assay also yielded similar results, the yeasts and bacteria were found more sensitive towards JRE followed by CCE. It was encouraging to see that in all the fungal and bacterial isolates growth was inhibited by JRE and CCE at different concentrations, and the halo was completely clear. The drug sensitivities for different *Candida* isolates tested by spot assay also revealed that cells show increased sensitivity to test extracts. In growth curve assays increase in concentration of test extract leads to significant decrease in growth with suppressed and delayed exponential phases with respect to control. In proteinase assay, at MIC/2 concentration of CCE and JRE, average decrease in proteinase secretion was 30.0 % and 44.4 %. Similar pattern was observed in case of phospholipase assay, MIC/2 concentration of CCE and JRE caused decreased in secretion by 25.0 % and 39.06 % respectively. In confocal microscopy, test extracts treated cells showed less viability as indicated by increased absorption of propidium iodide visualized by confocal scanning electron microscopy. Electron microscopy results showed extensive breakage in the cell wall and cell membrane. In toxicity assay CCE and JRE showed 8 % and 6 % hemolysis, while as fluconazole and tetracycline at their highest MIC of 32 & 16 µg/ml caused 20 % and 19 % hemolysis. **Conclusion**: Finally, plant derived extracts: CCE and JRE are found to be very active anti-candidal agents. JRE was most effective followed by CCE. Both of these test extracts severely affected growth, ultrastructure and tested pathogenicity parameters. These results taken together with the limited toxicity of methanolic extracts of CCE and JRE towards human red blood cells (6-8% lysis) make them eligible for further development as antifungals.