

Antifungal effect of *Punica granatum* L (pomegranate) peel and seed extracts and the effect of peel extract on the virulence factors of *Candida albicans*



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Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the Degree of Master of Science in Medicine.

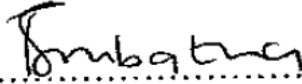
Johannesburg

2015

DECLARATION

I, Treasure Fundisiwe Mbatha declare that this dissertation is my own work. It is being submitted for the degree of the Master of Medicine to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

I declare that this thesis has the approval of The Committee for Research on Human Subjects (Medical). Ethical clearance certificate numbers M000402 and W-CJ-131101-1.

..... (Signature of candidate)

.....17..... Day ofSeptember.....2015

DEDICATION

In memory of my Mom and grandmother

Doreen Thobile Mbatha

(1968-2000)

Caroline Bikwaphi Mbatha

(1933-2013)

ABTRACT

Oral candidiasis which is commonly observed in immune compromised individuals, is caused by *Candida albicans*. Pathogenicity of *C. albicans* is dependent on virulence factors such as adherence to surfaces including host tissues, formation of hyphae and the production of hydrolytic enzymes. *C. albicans* isolated from HIV positive patients are known to express greater virulence and are considered to be more virulent than isolates from HIV negative patients. Antifungal drugs are available, but in recent years *C. albicans* has developed resistance to some of these drugs which has necessitated search for newer antifungal compounds. Medicinal plants can be an excellent resource for the discovery of new therapeutic drugs. *Punica granatum* (pomegranate) which is grown worldwide is known to have antimicrobial properties. Some studies have shown that at high concentrations

P. granatum has antifungal properties. However, high concentrations are difficult to maintain in body cavities with secretions. Therefore, this study investigated the antifungal properties of the peel and seeds of *P. granatum* and the effect of peel extract on the virulence properties of *C. albicans* isolated from HIV positive and HIV negative patients.

P. granatum was collected, fruit peel and seeds were separated, dried and pulverised. Extracts were prepared using various solvents and yields were calculated. Antifungal properties was determined against 10 strains of *C. albicans* isolated from the oral cavities of each of the HIV positive and HIV negative patients using a microdilution technique. Minimum inhibitory concentrations and minimum fungicidal concentrations were recorded. Subinhibitory concentration (3.125 mg/ml) of methanol extract of *P. granatum* was selected and its effect

on the adherence ability, germ tube formation and the production of phospholipase, proteinase and lipase by *C. albicans* was investigated using well described laboratory techniques.

For the fruit peel, methanol (0.67g/1g of dry powder) proved to be the best solvent for the crude extraction. For the seeds, hexane, dichloromethane/methanol and ethyl acetate produced the best yield (0.2 g/1g of powder). For the peel, minimal inhibitory concentration and minimal fungicidal concentration values of the ethanol, acetone, hexane, ethyl acetate and water against all the test isolates was 1.56 mg/ml. The lowest minimal inhibitory concentration and minimal fungicidal concentration of 0.39 mg/ml was obtained with ethyl acetate solvent. For the seeds, the median minimal inhibitory concentration and minimal fungicidal concentration values of the ethanol, acetone, hexane and water against all the test isolates was 1.56 mg/ml.

All the test isolates showed an adherence property to the oral epithelial cells. In the *C. albicans* isolated from HIV positive and HIV negative patients adherence was reduced by the plant extract from 331 to 321 cells ($p \geq 0.05$) and from 242.9 to 213.3 cells ($p \geq 0.05$) respectively. Germ tube formation by the *C. albicans* isolated from HIV positive patients and HIV negative patients was significantly reduced by the plant extract, from 68.7 to 51.1 cells (26% reduction – $p < 0.01$) and 72.4 to 38.4 cells (34% reduction – $p < 0.01$) respectively. In the presence of plant extract, the production of phospholipase by the *C. albicans* isolated from HIV positive patients and HIV negative patients either increased or decreased by 1 to 4% which was not significant. The plant extract reduced the production of proteinase by the *C. albicans* isolated from HIV positive patients and HIV negative patients by 2 to 3% which was also not significant. Similarly, production of lipase by the isolates from both the groups was reduced by 6 to 9% ($p \geq 0.05$). The reduction in the adherence ability, germ tube formation and the production of hydrolytic enzymes was not significantly different between the isolates from HIV positive and HIV negative patients.

High concentrations of crude extract of *P. granatum* peel have an antifungal effect and subtherapeutic concentrations can inhibit the germ tube formation which is necessary in the

pathogenesis of oral candidiasis. Therefore, pomegranate peel has a potential to be developed into a therapeutic agent, although further research is required.

PUBLICATIONS AND PRESENTATIONS

1. Poster presentation: Antifungal properties of *Punica granatum* (Pomegranate) against *Candida albicans*. Faculty Research Day, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, 2014.
2. Poster presentation: Antifungal properties of *Punica granatum* (Pomegranate) against *Candida albicans*. 6th Cross-Faculty Graduate Symposium, University of minimal inhibitory concentration and minimal fungicidal concentration the Witwatersrand, Johannesburg, South Africa, 2014.

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LIST OF ABBREVIATIONS AND ACRONYNS

API:	Profile Index
ATCC:	American Type Culture Collection
Als:	Agglutinin-like sequence
CFU/ml:	Colony Forming Units per millilitre
CHX:	Chlorhexidine Gluconate
CO ₂ :	Carbon Dioxide
<i>C. albicans</i> :	<i>Candida albicans</i>
<i>C. krusei</i> :	<i>Candida krusei</i>
<i>C. parapsilosis</i> :	<i>Candida parapsilosis</i>
Da:	Dalton
DMSO:	Dimethyl Sulphoxide
DNA:	Deoxyribonucleic Acid
g:	Gram
g/ml:	Gram per millilitre
GC-MS:	Gas chromatography- mass spectrometry
HPLC:	High performance liquid chromatography
HIV:	Human Immunodeficiency Virus
HWP1:	Hyphal wall protein 1
Hrs:	Hours
IgG:	Immunoglobulin G

kDa:	Kilo Dalton
LP/LIP:	Lipase
MFC:	Minimum Fungicidal Concentrations
MIC:	Minimum Inhibitory Concentrations
mg:	Milligram
mg/ml:	Milligram per millilitre
min:	Minutes
ml:	Millilitre
mM:	Millimolar
NMR:	Nuclear magnetic resonance
OD:	Optical Density
PBS:	Phosphate Buffered Saline
Ppm:	Parts per million
PL	Phospholipase
Pz:	Phospholipase activity
Pr:	Proteinase activity
PI:	Lipase activity
rpm:	Revolutions per minute
SAB:	Sabouraud dextrose agar
Saps:	Aspartic proteases
spp:	Species
TLC:	Thin Layer Chromatography
WHO:	World Health Organization
µg/ml:	Microgram per millilitre
µl:	Microlitre
µm:	Micrometre