Genomic and Proteomic Studies on the Antimicrobial Activity of *Teucrium polium* (Ja'adah) Extract Irradiated By Gamma Radiation

Thesis Submitted for Ph.D. degree in Microbiology

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Declaration

This thesis has not previously submitted for any other universities.

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SEIF ALDMOUR

ABSTRACT

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Teucrium polium was gamma irradiated using different doses and then, the antibacterial and antioxidant activities were evaluated. 20 kGy was the recommended dose according to antimicrobial activity (agar well-diffusion) against strains test (*Pseudomonas aeruginosa* and *Staphylococcus* aureus).

At 5.0 kGy, gamma irradiation has reduced bacterial community count in *T. polium* powder to 99.95%, and complete removal of total fungal count.

Further, based on the DPPH radical scavenging activity assay, *Teucrium polium* irradiated with 20kGy showed the highest scavenging activity.

The gas chromatography analysis of non-irradiated ethyl acetate *Teucrium polium* extract revealed a total of 48 compounds whereas in *Teucrium polium* irradiated with 20 kGy this number decreased to 34 compounds. Nevertheless, the content of 10 compounds increased after gamma irradiation while the content of 6 major compounds decreased.

The mechanism of *T. polium* antibacterial activity was investigated against *Pseudomonas aeruginosa* by estimating its effect on DNA fragmentation, scanning electron microscopy (SEM), antioxidant state and total protein.

The results revealed that *P. aeruginosa* when treated with non-irradiated or irradiated ethyl acetate *T. polium* extract showed a marked DNA fragmentation, while no fragmentation was observed in control (untreated cells). Also, SEM observation confirm the antibacterial activity of *T. polium*, as in both cases the bacteria lost its original shape and irregularity was observed.

Moreover, the results showed that, when *Pseudomonas aeruginosa* was treated with non-irradiated and irradiated (20 kGy) ethyl acetate *T. polium* extract there was a significant decrease in catalase activity, reduced glutathione concentration and increase in malondialdehyde compared to the control.

The mechanism of antibiofilm activity of non-irradiated and irradiated *T. polium* (20 kGy) was investigated against *P. aeruginosa*. The results revealed that non-irradiated and irradiated *T. polium* have significantly inhibited *P. aeruginosa* biofilm formation in a dose-dependent manner and have influenced the planktonic cell growth. Moreover, non-irradiated and irradiated *T. polium* have significantly down-regulated the expression of an important *P. aeruginosa* biofilm gene, *PslA, PelA*.

ABBREVIATIONS

List of abbreviations

Abb.	Description
%	Per cent
(V/V)	Volume per volume
$^{\circ}$C	Celsius
µg.	Microgram
^{60}Co	Cobalt-60
Å	Angstrom (10^{-10} meters)
A. fumigatus	*Asperegillus fumigatus*
A. pneumonia	*Actinobacter pneumonia*
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
B. subtilis	*Bacillus subtilis*
C. albicans	*Candida albicans*
CAT	Catalase
CDNA	Complementary deoxyribonucleic acid
CFU	Colony forming unit
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DPPH	2,2-Diphenyl-1-picrylhydrazyl
E. faecalis	*Enterococcus faecalis*
E. coli	*Escherichia coli*

EDTA	Ethylenediaminetetra-acetic acid
EPS	Extracelular polymeric substance
eV	Electron volt
g.	Gram
GC-MS	Gas Chromatography and Mass Spectroscopy
GSH	Reduced glutathione
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrogen chloride
hrs.	Hour/hours
IAEA	International Atomic Energy Agency
IC_{50}	Half maximal inhibitory concentration
kGy	Kilo Gray
L.	Litre
LPS	Lipopolysaccharid
m.	Meter
MDA	Malondialdehyde
mg.	Milligram
MIC	Minimum Inhibitory Concentration
min.	Minute
ml.	Milliliter
mm.	Millimeter

mmol	Millimole
NA	No activity
ng.	Nanogram
NIST	National Institute of Standards and Technology
nm.	Nanometer
nmol.	Nanomole
O.D	Optical Density
P. aeruginosa	*Pseudomonas aeruginosa*
PBS	Phosphate buffer solution
PCR	polymerase chain reaction
PelA	Pellicle A
PI	Percent Inhibition
PslA	polysaccharide synthesis locus A
PSM	Plant Secondary Metabolites
QPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic Acid
RNase	Ribonuclease
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
RT	Retention time
S. aureus	*Staphylococcus aureus*
SD±	Standard Deviation

SEM	Scanning Electron Microscope
SPSS	The Statistical Package for the Social Sciences
T. polium	*Teucrium polium*
TBC	Total Bacterial Count
TC	Tannins content
Temp.	Temperature
TFC	Total flavonoid content
TFC	Total Fungi Count
TLC	Thin layer chromatography
TPC	Total phenol content
UV	Ultra- Violet
WHO	World Health Organization
µl	Micro Littre
µm	Micrometer

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