In Vitro Anti-skin Cancer Properties and Mechanisms of Action of Xanthones from the Mangosteen Pericarp

A thesis submitted in fulfillment of the requirement for the degree of

Doctor of Philosophy

By

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Candidate’s Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

Jing Jing Wang

September 2012
Thesis Summary

The incidence of skin cancer has increased more than 600% worldwide since the 1940s, and Australians have the highest incidence in the world, with at least 2 in 3 Australians diagnosed with skin cancer before the age of 70. The current chemotherapy is not effective, with new drugs in high demand. Plants are important sources for anti-cancer drugs. Mangosteen (*Garcinia mangostana* Linn.) is a tropical tree from South East Asia and its fruit pericarp is a well-known traditional medicine.

This study investigated the potential anti-skin cancer activity of the crude extract and major xanthone compounds from the pericarp of mangosteen by investigating the cytotoxicity and underlying cellular and molecular mechanisms. Two types of human skin cancer cell lines were used as *in vitro* models: melanoma SK-MEL-28 and squamous cell carcinoma A-431.

There were five major research outcomes. (i) Development of a methodology for extraction of mangosteen based on chemical composition and antioxidant activity. (ii) Demonstration of anti-proliferative activity towards skin cancer cell lines. The crude extract and six xanthone compounds tested had significant anti-cancer activities, with *IC*₅₀ values ranging from 2.39 to 7.61 µg/ml. The activity was selective against skin cancer cells with less effect on human normal skin fibroblast CCD-1064Sk and the keratinocyte HaCaT cell lines. *IC*₅₀ values of the xanthones were similar to, or much lower than, those of two most commonly used commercial drugs (5-fluorouracil and dacarbazine). (iii) Identification of cellular and molecular pathways. The anti-cancer action of xanthone compounds was found to be via activation of caspases together with the loss of mitochondrial membrane potential.
and inhibition of Akt and NFκB survival pathways. In melanoma SK-MEL-28 cells, downregulation of BRAF V600E mutation expression was observed after treatment with some xanthones, e.g. a maximum 6.8-fold decrease in the level of BRAF V600E relative to the untreated control. (iv) Identification of synergistic effects. Synergistic effects between α-mangostin and the other individual compounds were observed. However, no synergistic effect was found between xanthone compounds and commercial drugs under the tested conditions in the current study. (v) Evaluation of anti-metastatic activity of α-mangostin. Skin cancers, especially melanoma, have a high potential to metastasise. α-Mangostin exhibited significant inhibitive activity of invasion and migration at non-toxic doses on both skin cancer cell lines tested. The anti-metastatic activity of α-mangostin was associated with downregulation of mRNA expression of MMP-2 and MMP-9 through inhibiting NFκB and Akt pathways.

This study provides important scientific evidence of the potential antioxidant and antiproliferative activity of extracts and xanthone compounds from the pericarp of mangosteen, and increases understanding of their underlying mechanisms. These findings can contribute to the development of novel plant-derived antioxidant strategies in the treatment of skin cancers.

**Keywords:** skin cancer; mangosteen; xanthones; cytotoxicity; apoptosis; survival pathway; metastasis
Acknowledgements and Dedication

First and foremost, I would like to pay principal acknowledgement to my supervisors, Prof. Wei Zhang and Dr. Barbara Sanderson, for their very great help in supervision of the project, my PhD candidature and the laboratory.

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In memory of my special close friend, Mrs. Alita Larsens.
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>AAPH</td>
<td>2, 2’-Azobis (2-amidinopropane) dihydrochloride</td>
</tr>
<tr>
<td>Akt</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>AU</td>
<td>Arbitrary unit</td>
</tr>
<tr>
<td>BRAF</td>
<td>Serine/threonine-protein kinase B-Raf</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree celcius</td>
</tr>
<tr>
<td>CDK</td>
<td>Cyclin-dependent kinases</td>
</tr>
<tr>
<td>CKI</td>
<td>Cyclin-dependent kinases inhibitors</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>Ct</td>
<td>Threshold of cycle</td>
</tr>
<tr>
<td>DAPI</td>
<td>4′, 6-Diamidino-2-phenylindole dihydrochloride</td>
</tr>
<tr>
<td>DMBA</td>
<td>7,12-dimethyl[a]benzanthracene</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPPH</td>
<td>2, 2-Diphenyl-1-picyrylhydrazyl</td>
</tr>
<tr>
<td>%DPPHRadSA</td>
<td>Percentage DPPH radical scavenging activity</td>
</tr>
<tr>
<td>DTIC</td>
<td>Dacarbazine</td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>EMEA</td>
<td>European agency for the evaluation of medicinal products</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FAK</td>
<td>Focal adhesion kinase</td>
</tr>
<tr>
<td>FBS</td>
<td>Foetal bovine serum</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric reducing antioxidant power</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-Fluorouracil</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic acid equivalents</td>
</tr>
<tr>
<td>GOI</td>
<td>Gene of interest</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HO⁻</td>
<td>Hydroxide ion</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>50% inhibitory concentration</td>
</tr>
<tr>
<td>IkB</td>
<td>Inhibitor of kappaB</td>
</tr>
<tr>
<td>IKK</td>
<td>IkB kinase</td>
</tr>
<tr>
<td>IL-8</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>IMDM</td>
<td>Iscoves Modified Dulbecco’s Medium</td>
</tr>
<tr>
<td>IU</td>
<td>International units</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun NH₂-terminal kinase</td>
</tr>
<tr>
<td>l</td>
<td>litre</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>µl</td>
<td>microlitre</td>
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</table>
Publications, Presentations and Awards

Publications

- Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang, 2011. Cytotoxic effect of xanthones from pericarp of the tropical fruit mangosteen (Garcinia mangostana Linn.) on human melanoma cells. Food and Chemical Toxicology. 49: 2385–2391


- Jing J. Wang, Qing H. Shi, Wei Zhang, Barbara J.S. Sanderson, 2012. Anti-skin cancer properties of phenolic-rich extract from the pericarp of mangosteen (Garcinia mangostana Linn.). Food and Chemical Toxicology. 50: 3004-3013.

Publications in Submission

- Jing J. Wang, Wei Zhang, Barbara J.S. Sanderson, 2012. Altered mRNA expression related to the apoptotic effect of three xanthones on human melanoma SK-MEL-28 cell line. Food and Chemical Toxicology (Under review; manuscript No. FCT-6676).

Publications in preparation


- Jing J. Wang, Wei Zhang, Barbara J.S. Sanderson. Xanthones isolated from mangosteen pericarp induce apoptosis in human melanoma SK-MEL-28 cells.

- Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang. Potential synergistic skin cancer suppression by combination of xanthones.

Presentations

- ASMR SA Scientific Meeting 6th June 2012. Adelaide, Australia. Oral Presentation "Inhibitory effect of α-mangostin on proliferation and metastasis of human melanoma SK-MEL-28 cell line"


Poster Presentation. “Anti-skin cancer activity of crude extract of mangosteen (Garcinia mangostana Linn.)”


- 13th World Congress on Cancers of the Skin. 7th -10th April 2010. Madrid, Spain. Oral Presentation “Evaluation of antiproliferation properties of xanthones from pericarp of mangosteen (Garcinia mangostana L.) on human melanoma cells”

Awards

- 2012 Ross Wishart Memorial Award finalist
- 2011 AusBiotech-GSK Student Excellence Award – State Winner
- 2011 Chinese Government Award for Outstanding Self-financed Students Abroad
- 2010 “Top Ten Cited Author in 2007 & 2008” from Mutation Research
- 2010 AusBiotech-GSK Student Excellence Award – State Finalist
- 2008 AusBiotech-GSK Student Excellence Award – State Finalist
- 2008 – 2012 EPRIS Scholarship, Flinders University, Australia

Professional Membership

- 2010- current AusBiotech (Australia’s Biotechnology Organisation)
- 2012 – 2013 Australian Society for Medical Research
- 2012-2013 Bioprocessing Network
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A11.8.2 Amplification efficiency (AE)

A11.9 One example of raw data of qRT-PCR standard curve

A11.10 qRT-PCR melt curve for each gene

A12 Cytotoxicity of α-mangostin at low concentrations on A-431 and SK-MEL-28 cells
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