ANALYSIS OF THE POTENTIAL FOR *RUBUS* FRUIT ELLAGITANNINS TO INDUCE ANTI-INFLAMMATORY EFFECTS IN *IN VITRO* MODELS

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Abstract

Nutrigenomics is the study of interactions between human diets and the genome with the aim of discerning how food components influence gene and protein expression in individuals. Previous studies have established the potential for ellagitannins (ETs) isolated from various fruits, to induce anti-inflammatory responses that may be beneficial to those afflicted with Inflammatory Bowel Disease (IBD).

The hypothesis of this thesis is that *Rubus* fruit extracts, rich in ETs, induce an anti-inflammatory effect in mammalian cells involved in inflammatory processes.

Methods were developed to extract, purify, and quantify the phytochemical composition of three selected *Rubus* fruit cultivars (HB19, Wakefield and ZZ).

Extracts (EX: phenolic extract, ETx: ET-enriched extract) were initially assessed using a secondary cell line: RAW 264.7 macrophages. Cell viability using the MTT assay, found no cytotoxic effect at polyphenolic concentrations up to 100 µg/mL. The Griess assay measured levels of nitric oxide (NO) production and found a decrease at 50 µg/mL of polyphenolic exposure for most extracts except ZZ EX. Cytokine assessment showed that the extract, Wakefield ETx, lowered production of Interleukin 6 (IL6) by macrophages. All extracts increased production of Tumour Necrosis Factor alpha (TNFα) by macrophages.

Colonic intestinal epithelial cells (colonic IEC) and bone marrow derived macrophages (BMDM), were isolated from control C57BI/6J mice and Interleukin 10 gene-deficient mice (*Il10*$^{-/-}$). Viability results suggested that the polyphenolic concentration of 50 µg/mL, was the highest tolerated concentration for NO and cytokine assays. NO production was decreased for BMDM (*Il10*$^{-/-}$) with extracts Wakefield EX and ZZ EX, and for BMDM (C57) with most extracts except HB19 EX. IL6 production by colonic IEC (*Il10*$^{-/-}$) was decreased for all extracts. The cytokine profile for BMDM (*Il10*$^{-/-}$) mice showed a decrease in IL12 and IL6 production, and no change in TNFα production in response to co-incubation with all extracts.

There are indications from research done in this thesis that the *Rubus* fruit extracts from “ZZ” and “Wakefield” cultivars reduce inflammatory immune responses or potentially having the ability to act on different pathways or induce a wound healing response.

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Abbreviations Used

Abbreviation	Full meaning
BMDM	Bone marrow derived macrophages
C57	C57BI/6J mice
CA	Cellulose acetate
CD	Crohn's Disease
CFU	Colony forming units
CIF	Conventional intestinal flora
COX2	Cyclooxygenase-2
Complete BRPMI	Complete Bone Marrow RPMI-1640
Complete IRPMI	Complete IEC RPMI-1640
DM	Dry matter
Complete DMEM	Liquid high-glucose Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DSS	Dextran sodium sulfate
DTT	Dithiothreitol
DTT HBSS	HBSS 1% Pen/Strep with 15 mM DTT
EA	Ellagic acid
EGF	Epidermal growth factor
ES	*Enterococcus* suspension
ET	Ellagitannin
ETx	*Rubus* fruit ellagitannin enriched extract
EX	*Rubus* fruit phenolic
FBS	Foetal bovine serum
FW	Fresh weight
GHS	General Health Score
GIT	Gastrointestinal tract
GWAS	Genome-wide association studies
HBSS	Hanks' Balanced Salt Solution
HETx	HB19 ellagitannin extract
HEX	HB19 phenolic extract
HHDP	Hexahydroxydiphenoyl
HPLC	High-performance liquid chromatography
IBD	Inflammatory Bowel Disease
IEC	Intestinal epithelial cells
IFNγ	Interferon γ
IL	Interleukin cytokine (family group name)
Il10$^{-/-}$	IL10 gene-deficient mice
Lam C	Lambertianin C
LCMS	Liquid chromatograph-mass spectrometry
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinases
M-CSF	Macrophage colony stimulating factor
MTT	MTT assay
NED	Naphthylenediamine dihydrochloride
NF-κβ	Nuclear factor-κβ
NO	Nitric Oxide
NOD2	Nucleotide oligomerisation domain 2

PBS	Phosphate buffered saline
PE	Pomegranate extract
Pen/Strep	Penicillin and Streptomycin
Plant & Food Research	The New Zealand Institute for Plant & Food Research Limited
RPMI	Liquid Roswell Park Memorial Institute medium
RT	Room temperature
SH6	Sanguiin-H6
SNP	Single nucleotide polsymorphisms
T_h 1	T helper 1
TLR	Toll-like receptor
TNFα	Tumour Necrosis Factor alpha
UC	Ulcerative Colitis
Uro	Urolithin
WETx	Wakefield ellagitannin extract
WEX	Wakefield phenolic extract
WST-1	WST-1 assay
ZETx	ZZ ellagitannin extract
ZEX	ZZ phenolic extract