Phenolics and Condensed Tannins of Forage Plants from Botswana and their Biological Significance

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Olekile Tibe
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ABSTRACT

Phenolics and Condensed Tannins of Forage Plants from Botswana and their Biological Significance

By Olekile Tibe

Supervised by Professor David R.K. Harding, Dr Ian Sutherland and Dr Lucy Lesperance
Institute of Fundamental Sciences, College of Sciences,
Massey University, Palmerston North

The main objectives of this study were to isolate and characterise phenolics and CT from Botswanan forage plants, and to investigate their potential anti-parasitic and immunostimulatory properties. *Viscum rotundifolium, Viscum verrucosum, Tapinanthus oleifolius, Grewia flava and Ipomoea sinensis* forage leaves and small stems were harvested over two summers (February 2009 and 2010), extracted twice with acetone:water (7:3) and subsequently defatted with dichloromethane. Each crude extract (6 g) was loaded onto a Sephadex LH-20 column and eluted with aqueous methanol (1:1) to yield four fractions, and subsequently eluted with acetone:water (7:3) to yield three fractions. Phytochemical screening of the fractions for the presence of CT and phenolics was conducted by a reverse phase high performance liquid chromatography coupled to a photodiode array (RP-HPLC-PDA) detector at 280 nm. The butanol-HCl colorimetric assay revealed that the total CT concentrations from the forage plants ranged from 0.2 to 12.7 (g/100g dry matter). These results indicated that significant amounts of CT were present in *V. verrucosum*, *T. oleifolius* and *G. flava*.

The potential impact of each purified CT fraction was evaluated for anti-parasitic effects using a larval development assay (LDA). Three different species of gastrointestinal nematodes (*Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*) from sheep were tested with the fractions at 100 and 500 μg/mL. CT fractions from *V. rotundifolium* and *I. sinensis* samples, collected in 2009 and 2010, did not inhibit larval development (L1 and L2) to the infective L3 stage. CT isolated from *V. verrucosum* and *T. oleifolius* which were collected in 2009 completely inhibited the development of all parasite species at both concentrations. Also, complete inhibition of larval development of all tested parasite species was obtained in CT fractions from *G. flava* collected in 2009, with the lowest inhibitory activity at 62.5 μg/mL. These findings suggest that CT extracts have anti-parasitic effects *in vitro*, which may be translated into reduction of the effects of parasitism in ruminants *in vivo.*

The potential impact of the extracts on priming of γδ T cells from the peripheral blood of lambs, calves and kids at 5 and 10 μg/mL was also evaluated *in vitro*. Condensed tannins (CT) from *G. flava* significantly primed γδ T cells in kids by up to 64.75% at 10 μg/mL, which was statistically significant relative to the negative control at 22.66% (p=0.004). CT from *T. oleifolius* also induced priming of γδ T cells in kids, while fractions from *V. rotundifolium* and *V. verrucosum* induced minimal priming of γδ T cells. These findings suggest that CT from a selected Botswanan forage plants can stimulate the immune system *in vivo* in selected ruminant species.

The anti-parasitic and immunostimulatory effects could be influenced, among other things, by the chemical structure and concentration of CT in the forage sample. The ^{13}C-NMR and thiolysis results revealed that CT from *V. verrucosum, T. oleifolius* and *G. flava* were procyanidin (PC) and *cis* dominant. Further purification of the thiolysis adducts of CT from these plants led to the isolation of (-)-epicatechin which was found to be the dominant compound in the extension units of the CT polymer. The final stage of the research was the re-chromatography of methanolic fractions containing low molecular weight phenolics. The low molecular weight phenolics from the methanolic fractions were successfully purified and isolated. The characterisation of flavonoids by NMR and LC-ESI-MS/MS showed that quercetin was predominant in the purified fractions with attached sugars such as rhamnose, glucose and apiose.

Publications

Part of this project including the sulla research related to my PhD project has been published in:

1. **Tibe**, **O**., Lesperance, L., Fraser, K., and Harding D.R.K (2011). Condensed tannins and flavonoids from sulla (*Hedysarum coronarium*). *Journal of Agricultural and Food Chemistry*, 59 (17), 9402-9409.

2. **Tibe**, **O**., Sutherland, I., Lesperance, L., Pernthaner, A., and Harding, D.R.K (2012). Condensed tannins from Botswanan forage plants are effective priming agents of gamma delta γδ T cells in ruminants. *Veterinary Immunology and Immunopathology*. 146 (2), 237-244.

3. **Tibe, O.**, Sutherland, I., Lesperance, L., and Harding D.R.K (2012). The effect of condensed tannins from Botswanan forage plants on the free-living stages of gastrointestinal nematode parasites of livestock. *Veterinary Parasitology* Journal. In press.

One paper in preparation is to be published

1. **Tibe, O.**, Sutherland, I., Lesperance, L., and Harding D.R.K (2012). Condensed tannins and flavonoids from Botswanan forage plants. *Phytochemistry* Journal.

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LIST OF ABBREVIATIONS

aq	Aqueous
APCI	Atmospheric pressure chemical ionisation
AWMT	Adult worm motility test
BCA	Botswana College of Agriculture
Bu	Butanol
C-18	HPLC column coated with a carbon 18 reverse phase
C	Catechin
CCA	R-cyano-4-hydroxycinnamic acid
COSY	Correlated spectroscopy
CSA	5-chlorosalicylic acid
CCC	Countercurrent chromatography
CT	Condensed tannins
d_4-MeOH	Deuterated methanol
d_6-acetone	Deuterated acetone
d	Doublet (spectral)
Da	Daltons
DAMCA	Dimethylamino-cinnamaldehyde
DCM	Dichloromethane
DEPT	Distortionless enhanced by polarisation transfer
DHB	2,5-dihydroxybenzoic acid
DM	Dry matter
EC	Epicatechin
EGC	Epigallocatechin
EHA	Egg hatch assay
ESI-MS	Electrospray Ionization Mass Spectrometry
FTIR	Fourier transform infrared
GC	Gallocatechin or gas chromatography
GIN	Gastrointestinal nematodes
^{1}H NMR	Proton (hydrogen). In reference to NMR spectroscopy
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HSQC	Heteronuclear single quantum correlation

HPLC	High performance liquid chromatography
HT	Hydrolysable tannin
IAA	trans-3-indolacrlylic acid
IR	Infrared spectroscopy
IS	Internal standard
ISA	Immunostimulatory assay
J	Coupling constant (in NMR spectrometry)
LC	Liquid chromatography
LCS	*Lotus corniculatus*
LDA	Larval development assay
LMI	Larval migration inhibition
LMW	Low molecular weight
LP	*Lotus pedunculatus*
MHC	Major histocompatibility complex
MALDI-TOF	Matrix aided laser desorption ionization, time of flight
MeOH	Methanol
mDP	Mean degree of polymerisation
MIP	Molecularly imprinted polymers
MMW	Medium molecular weight
Min	Minute
mL/min	Millilitre per minute
mmol	Millimole
mol	Mole (s)
mol/L	Moles per litre
MS	Mass spectrometry
Mw	Molecular weight
NA	9-nitroanthacene
NDF	Neutral detergent fibre
NIRS	Near infrared reflectance spectroscopy
nm	nanometer
NMR	Nuclear magnetic resonance
NP-HPLC	Normal phase high pressure liquid chromatography
ODS	Octadecyl silica
PA	Proanthocyanindins

PC	Paper chromatography
PCs	Procyanidins
PDs	Prodelphinidins
PDA	Photodiode array detector
PEG	Poly(ethylene glycol)
RP-HPLC	Reverse phase high pressure liquid chromatography
RRF	Relative response factor
s	Singlet (spectral)
SA	Sinapinic acid
SPME	Solid phase microextraction
t	Triplet (spectral)
TCR	T cell receptors
thio-	thiolysis adduct
TLC	Thin-layer chromatography
TOCSY	Total correlated spectroscopy
UV-Vis	Ultra violet-visible