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Antioxidant properties and cellular protective effects of selected African green leafy vegetables

by

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DECLARATION

I hereby declare that the thesis which I herewith submit at the University of Pretoria for the award of PhD degree (Nutrition) is my research and has not been submitted by me for a degree at any other university or institution of higher learning.

Nangula Paulina Mavhungu

14 November 2011

“perseverance, character; and character, hope.”

Romans 5:4 (NIV)

ABSTRACT

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By

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Supervisor: Prof. A. Oelofse
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Phenolic compounds in African green leafy vegetables (GLVs) may have a significant impact on human health. However, there is little information on the phenolic composition, antioxidant properties, as well as biological and cellular protective effects of these vegetables. The effects of boiling and extraction solvent on these compounds and on their antioxidant properties are also unknown.

Phenolic content, antioxidant activity and cellular protective effects of four African GLVs in comparison with spinach, an exotic GLV, was determined. African GLVs had appreciable levels of total phenolics and antioxidant activity and in higher quantities compared to spinach. Boiling decreased the antioxidant content and activity of these vegetables and 75% acetone was more effective in extracting antioxidants from the GLVs compared to water. GLVs with high levels of phenolics also contained higher levels of antioxidant activity, suggesting that phenolics are likely to have contributed to radical scavenging activity of these vegetable extracts, even though the degree of scavenging varied in each extract of the vegetable species.

The flavonoid compositions of raw and boiled African GLVs and spinach were determined using high-performance liquid chromatography. Epicatechin and rutin were the most dominant flavonoids found in both water and 75% acetone extracts. Among water extracts, pumpkin contained higher concentrations of detected flavonoids, while among the acetone extracts, cowpea exhibited higher concentrations. The effect of boiling was dependent on the type of vegetable and the specific flavonoids. There were no major differences observed between the type of flavonoids detected in extracts of African GLVs and those in spinach. However, similar to the results of total phenolics and antioxidant activity, the 75% acetone extracts of African GLVs also exhibited higher amounts of flavonoids than spinach.

The protective effects of GLVs against oxidative haemolysis were dependent on the type of vegetable species. Boiling had variable effects depending on the species. The highest level of protection of erythrocytes against oxidative damage was offered by amaranth extracts, while extracts of raw jute mallow contributed to the damage of erythrocytes. The highest antioxidant protection activity against oxidative damage in plasmid DNA was offered by extracts of jute mallow and lowest by spinach.

For the cell viability assays, GLVs were evaluated to determine their cytotoxicity levels and functional role in oxidative damage. The results of the long-term cell viability (i.e. MTT, NR and CV) assays indicated no cytotoxicity, while the short-term cell viability (i.e. DCF) assay indicated that all extracts of raw GLVs were significantly ($p < 0.05$) cytotoxic to SC-1 fibroblast and human adenocarcinoma colon cancer (Caco-2) cells than extracts of cooked samples, and the levels of toxicity in the extracts of spinach was higher than in African GLVs. These results indicate that there was an initial cytotoxic effect as extracts of raw GLVs were added to the cells. However, after about 72 h, the cells recovered from the initial shock and started proliferating as usual. In the presence of peroxyl radicals, extracts of African GLVs exhibited higher protective effects against oxidative damage in both types of cell cultures than extracts of spinach. These results indicate that these protective effects could be attributed to the presence of phenolics and antioxidant properties of these extracts.

Although boiling reduced the antioxidant content and activity of African GLVs, the levels remained higher than in spinach. Boiling also decreased the cytotoxicity and cell damage caused by extracts of raw GLVs samples. African GLVs are consumed after boiling, and therefore the observed cytotoxicities might not be experienced in practical terms. African GLVs have therefore a potential to reduce the risk and development of diseases associated with oxidative stress in communities that consume these vegetables.

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GLOSSARY

AAPH	2,2′-azobis(2-amidinopropane) dihydrochloride
ABTS	2,2′-Azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt
ADME	absorption, distribution, metabolism and excretion
ANOVA	analysis of variance
approx	approximately
ARC	Agricultural Research Council
AUC	area under the fluorescence curve
°C	degree Celsius
C	carbon
Caco-2	human adenocarcinoma colon cancer
CDL	chronic diseases of lifestyle
CH_3O	methoxyl
CHD	coronary heart diseases
CO_2	carbon dioxide
Cu	copper
CV	crystal violet
CVD	cardiovascular diseases
DCF	dichlorofluorescein
DCFH	dichlorofluorescin
DCFH-DA	dichlorofluorescein diacetate
DMEM	Dulbecco's modified eagle medium
DMPD	*N*,*N*-dimethyl-*p*-phenylelendiamine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-2-picrylhydrazyl
dw	dry weight
EDTA	ethylenediaminetetracetric acid
EGCG	epigallocatechin gallate
EtBr	ethidium bromide

FAO	Food and Agriculture Organization of the United Nations
F-C	Folin Ciocalteu
FCS	Fetal calf serum
Fe	iron
Fig	Figure
fw	fresh weight
FRAP	ferric reducing antioxidant power
g	gram
GAE	gallic acid equivalents
GC/MS	gas chromatography combined with mass spectrometry
GLVs	green leafy vegetables
GSH	glutathione peroxidase
H	hydrogen
h	hour
H_2O_2	hydrogen peroxide
HAT	hydrogen atom transfer
HCl	hydrochloric acid
HIV/AIDS	human immunovirus / acquired immune deficiency syndrome
HOBr	hypobromous acid
HOCl	hypochlorous acid
HOO	hydroperoxyl
HORAC	hydroxyl radicals averting capacity
HPLC	high performance liquid chromatography
HPLC/DAD/MS	high performance liquid chromatography equipped with a diode array detector and mass spectrophotometer
i.e.	that is
kcal	kilo calories
KCl	potassium chloride
kg	kilogram
kJ	kilo joules
$K_2S_2O_8$	potassium peroxodisulfate

L	litre
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LPH	lactase phloridzin hydrogenase
LSD	least significant difference
M	molar
mg	milli gram
ml	milli litre
min	minutes
mM	milli molar
MTT	3,(4,5-dimethyl thiazol-2-yl)2,5-diphenyl tetrazolium bromide
n	number of flavonoid units
NaCl	sodium chloride
Na_2EDTA	ethylene diamine tetra acetic acid disodium salt dehydrate
NaH_2PO_4	sodium phosphate monobasic
Na_2HPO_4	di-sodium hydrogen orthophosphate dehydrate
n.d.	not determined
n.d.a.	no data available
nm	nano mitre
NO^-	nitric oxide
NO_2^-	nitrogen dioxide
NR	neutral red
O	oxygen
O_2^-	superoxide
OD	optical density
OH^-	hydroxyl ion
OH	hydroxyl
ONOO	peroxynitrite anion
ORAC	oxygen radical absorption capacity
pBR	plasmid Boliver and Rodrigues
PBS	phosphate buffer solution

PCL	photochemiluminescence
pH	potential hydrogen
RE	retinol equivalents
$RO^{\cdot}$	alkoxyl
$ROO^{\cdot}$	peroxyl
ROS	reactive oxygen species
SACN	Scientific Advisory Committee on Nutrition
SD	standard deviation
SET	single electron transfer
SEM	standard error of means
SOD	superoxide dismutase
sp.	species
TAA	total antioxidant activity
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
TFC	total flavonoid content
TIFF	tagged image file format
TPC	total phenolic content
TRAP	total radical-trapping antioxidant parameter
USA	United States of America
USDA	United States Department of Agriculture
UV	ultra violet
WHO	World Health Organization
µg	micro gram
µl	micro litre
µM	micro molar
µmol	micro moles