in vivo *26*: 99-106 (2012)

Suppression of Caco-2 and HEK-293 Cell Proliferation by *Kigelia africana*, *Mimusops zeyheri* and *Ximenia caffra* Seed Oils

ELITON CHIVANDI[1], ELEANOR CAVE[1], BRUCE CLEMENT DAVIDSON[2], KENNEDY HONEY ERLWANGER[1], DAVISON MOYO[1] and MICHAEL TAURAI MADZIVA[1]

[1]*School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Republic of South Africa;*
[2]*Saint James School of Medicine, Bonaire, Dutch Caribbean*

Abstract. *Animal-derived oils and purified fatty acids, but not indigenous fruit-tree-derived seed oils, have been used to study cell growth and differentiation. In this study, we determined the effects of the Kigelia africana, the Mimusops zeyheri and the Ximenia caffra seed-oil on cell proliferation in culture. Human colon adenocarcinoma (Caco-2) and human embryonic kidney (HEK-293) cells were maintained and treated with various concentrations (0, 20, 40, 80, 100 and 120 mg/l) of K. africana, M. zehyeri and X. caffra seed oil. The trypan blue dye exclusion method was used to determine cell growth 48-hours after oil treatment. All three tree seed oils suppressed both Caco-2 and HEK-293 cell growth in a dose-dependent manner. Importantly, the tree seed oils did not cause increased cell death as the number of dead cells remained unchanged under control and oil-treated conditions. K. africana oil significantly suppressed Caco-2 cell growth compared to HEK-293 cell growth at all oil concentrations, whereas M. zeyheri and X. caffra seed oils significantly suppressed HEK-293 and Caco-2 cell growth, only at a concentration of 80 mg/l. The suppression of Caco-2 and HEK-293 cell proliferation by K. africana, M. zeyheri and X. caffra seed oils suggest a potential antiproliferative effect of these tree seed oils on the two cell lines.*

Research on indigenous fruit-bearing trees (IFBTs) is largely focused on the medicinal and pharmacological potential and the phytochemical composition of the tree leaf, root and stem bark extracts and to some extent fruit pulp composition. Seeds from IFBTs are largely discarded after utilisation of their fruit pulp despite their being potential sources of nutrients and oils. Our laboratory has embarked on a major study to characterize the seeds of some prominent IFBTs in the sub-Saharan African region. We decided to determine the potential of *Kigelia africana* (family Bignoniaceae), *Mimusops zeyheri* (family Sapotaceae) and *Ximenia caffra* (family Olacaceae), which are widely distributed in the sub-Saharan Africa region (1-3), as food and feed ingredients. *M. zeyheri* and *X. caffra* supply edible fruit that are rich in ascorbic acid (4, 5); *K. africana* fruit in Malawi is used to aid fermentation in traditional beer brewing and the seeds are roasted and eaten in times of famine (6).

Our work so far has proved the beneficial energy and protein profiles of *X. caffra* (7), *M. zeyheri* (8) and *K. africana* (9) seeds. Furthermore, the seeds have high oil yields (48.50%, 23.38% and 49.22%, respectively) compared to most conventional oil seed crops. Seed oils from the three IFBTs seeds have fatty acid profiles that indicate their potential as sources of essential fatty acids and for possible use as edible oils, in pharmaceuticals and as raw materials for industrial detergents and lubricants. However, plant seed oils at times contain liposoluble toxic compounds, such as tumor-promoting phorbol esters found in *Jatropha curcas* seed oil (10), the toxic gossypol found in crude cotton (*Gossypium hirsutum*) seed oil (11) and the encephalopathy-causing Neem seed oil (12).

Biological characterization of oils using of cells in culture has largely focused on animal-derived oils and purified fatty acids while little if any investigations have been carried out on seed oils from IFBTs. Given their natural content the seeds of IFBTs could be used to generate non-conventional feed ingredients in the form of oil seed cakes and meals. However, due to their high oil content, the oil would firstly have to be extracted, yet there is usually residual oil in the seed cake even after oil extraction, which could cause deleterious effects on animals if the seed cakes are used as feed ingredients. We therefore undertook a

Correspondence to: Eliton Chivandi, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193, Johannesburg, Republic of South Africa. Tel: +27 117172567, Fax: +27 116432765, e-mail: Eliton.Chivandi@wits.ac.za

Key Words: Indigenous fruit-bearing tree seed oils, Caco-2 cells, HEK-293 cells, growth suppression, essential fatty acids, *Kigelia africana, Mimusops zeyheri, Ximenia caffra*.

preliminary screening study of the three IFBTS seed oils *in vitro* using human colon adenocarcinoma cells (Caco-2), a commonly used gastrointestinal cell line, and human embryonic kidney (HEK-293), an epithelial immortalised cell line. Caco-2 cells are known to form tight junctions (13-14), have structural properties similar to those of ileal enterocytes (15) and are hence useful models as they mimic the gastrointestinal tract. The HEK-293 cell line has been used extensively (16-17) as a laboratory work-horse in several cell culture studies. The objective of this study was to determine the effect of *K. africana*, *M. zeyheri* and *X. caffra* seed oils on Caco-2 and HEK-293 cell proliferation.

Materials and Methods

Seed source and oil extraction. Ripe *K. africana* fruits were harvested from trees in Gutu District, Zimbabwe. The area lies within latitude and longitude 19˚41'S and 31˚09'E, is characterized by granitic soils, with annual rainfall and temperature ranges of 650-800 mm and 20.5˚C to 30.0˚C, respectively (18). Fresh ripe *M. zeyheri* fruits were harvested from trees in Gokwe District, Zimbabwe (18˚13'S and 28˚56'E) characterized by granitic deep gusu sandy soils, an average annual rainfall of 819 mm and an annual average temperature of 26˚C (19). Ripe *X. caffra* was collected from Zhombe District (latitude 14˚45'S and longitude 26˚50'E) in the Midlands province of Zimbabwe. Zhombe District is characterized by low annual rainfall with a mean of 550 mm and a mean temperature of 26˚C. Representative samples of ripe fruit from 20 trees of each species were collected as follows: 10 fruits from each *K. africana* tree, and a 100 fruits from each of the *M. Zeyheri* and *X. caffra* trees. Each *K. africana* fruit yields more seeds compared to the fruits from the other two IFBTs species. Following extraction from the fruit, the seeds of each tree species were thoroughly mixed and dried under shade. Twenty percent of the dried seeds of each tree species were then stored in brown sample bottles at 4˚C to minimize oxidation of oils in the seeds. Prior to extraction of the oil at the University of the Witwatersrand, the seeds were hand shelled. The shelled seeds were then crushed in a blender (Lasec, Pty. Ltd., Johannesburg, South Africa) to generate the three respective compound seed meals out of which the seed oils were extracted and stored at –20˚C prior to use.

Fatty acid profile determination. For the *K. africana* and *M. zeyheri* seeds, oil was extracted by the Soxhlet method. Methyl esters were prepared for capillary gas chromatography according to the method of Christopherson and Glass (20). Briefly, the fat extracts were trans-methylated with 2M methanol-sodium hydroxide. The resulting fatty acid methyl esters were extracted in heptane, filtered and dried under nitrogen. The fatty acids were separated by a temperature gradient over 45 min on an HP6890 GC (Hewlett Packard, Bristol, UK) with nitrogen as the carrier gas on a DB-23 capillary column (90 cm × 250 μm × 0.25 μm) (Supelco, Sigma-Aldrich, Bellefonte, Pennsylvania, USA). Lipid extraction and oil purification from *X. caffra* seed meal was carried out as described by Bligh and Dyer (21) with methyl esters of fatty acids being prepared using 10% acetyl chloride in methanol with incubation at 50˚C overnight. The resultant methyl esters were extracted into hexane and separated on a Varian gas chromatograph isothermally at 195˚C with a 10% SP2330 on a chromosorb 100/120 WAW 2 m × 3.2 mm column and quantified using flame ionisation detection and a Varian 4270 integrator.

Cell culture. The Caco-2 and HEK-293 cell lines used in the study were obtained from Highveld Biologicals (Pty) Ltd., Johannesburg, South Africa. Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum (FBS) used to maintain the two cell lines were obtained from Highveld Biologicals (Pty) Ltd., Johannesburg, South Africa. Each cell line was grown in DMEM containing 10% FBS (v/v) in 75 cm^2 flasks and incubated in an Autoflow IR-Water-Jacketed CO_2 Incubator [Lasec SA (Pty) Ltd, Johannesburg, South Africa] under 10% CO_2 at 37˚C. To maintain the characteristics of each cell line, cells were passaged (inside an ESCO Class II Biosafety Cabinet; ESCO, Johannesburg, South Africa) at less than 80% confluence and put in fresh DMEM containing 10% FBS.

Oil treatment and viability determination. Prior to dosing of the cells with the seed oils, any contaminants in the oils were removed by microfiltration. The micro filtered seed oils were solubilised in 99.5% ethanol (Merck Chemicals, Johannesburg, South Africa). A pilot study (at the maximum solubilisation dose) indicated that the ethanol itself at this specific concentration had no effect on cell viability (data not shown). The seed oil solutions were prepared to 0, 20, 40, 80, 100 and 120 mg oil/l final oil concentrations per culture plate well. Ethanol (99.5 % at 120 mg/l) was used in the control wells. Cells were lifted from the flasks using phosphate- buffered saline containing 1 mM EDTA.

Cells in medium were seeded into 24-well tissue culture plates at 1×10^5 cells per well followed by addition of 1 ml of medium (DMEM containing 10% FBS), and then incubated in an Autoflow IR-Water-Jacketed CO_2 Incubator [Lasec SA (Pty) Ltd] under 10% CO_2 at 37˚C for 24 hours to recover prior to treatment (dosing) with the oils. Following the 24-h recovery period, cells in each well were individually dosed, inside an ESCO Class II Biosafety Cabinet (ESCO), with the appropriate tree seed oil (treatments) at the requisite concentrations. Each treatment dose of the respective seed oils was replicated three times for each cell line. After application of the oils, the cells were incubated for 48 hours (22, 23). The trypan blue exclusion method (24) was then used to assess cell viability.

Statistical data analysis. An analysis of variance using the GraphPad Prism 5 statistical package (GraphPad Software, San Diego, California, USA) was performed and Bonferroni's *post hoc* test was used to compare the effects of each tree seed oil on the two cell lines at the same oil concentration level and to the control.

Results

Lipid yield and fatty acid profiles of the IFBT seed oils. *K. africana* had the highest oil yield (49.2%) with the highest content of α-linolenic acid (54.29%), whilst *M. zeyheri* had the lowest oil yield (23.4%) with the highest linoleic acid content at 23.86% of the seed oil (Table I).

Effect of the IFBT seed oils on Caco-2 and HEK-293 cells. The number of non-viable (dead) cells of different treatments and in comparison to the control was low, and more importantly, there was no statistically significant difference (data not shown) in the number of dead cells across treatments compared to the control, an indication that the oils did not cause cell death. Figures 1-3 show the effect of *K. africana*, *M. zeyheri* and *X. caffra* seed oils on Caco-2 and HEK-293

Table I. *Lipid yield (%) and fatty acid composition (%) of the tree seed oils.*

Parameter	*K. africana*	*M. zeyheri*	**X. caffra*
Lipid yield	49.22	23.38	48.5
Fatty acid profile			
Saturated			
C12:0 (lauric acid)	0.03±0.00	0.15±0.12	nd
C14:0 (myristic acid)	0.05±0.02	0.13±0.02	0.02±0.01
C15:0 (pentadecanoic acid)	nd	0.04±0.01	nd
C16:0 (palmitic acid)	7.54±0.25	14.06±0.01	1.47±0.20
C17:0 (margaric acid)	0.11±0.01	0.12±0.01	nd
C18:0 (stearic acid)	4.56±0.15	6.92±0.01	0.45±0.10
C20:0 (arachidic acid)	0.89±0.02	0.73±0.00	nd
C21:0 (heneicosanoic acid)	0.22±0.28	0.09±0.10	nd
C22:0 (behenic acid)	0.43±0.02	0.32±0.01	0.56±0.24
C23:0 (tricosanoic acid)	0.03±0.02	0.03±0.01	nd
C24:0 (lignoceric acid)	0.23±0.02	0.18±0.00	17.84±1.46
TSFA	14.10±0.23	22.73±0.08	20.19±1.07
Monounsaturated			
C14:1n7 (myristoleic acid)	nd	0.01±0.00	0.03±0.01
C16:1n7 (palmitoleic acid)	0.10±0.07	0.08±0.01	nd
C17:1 n8 (8-heptadecenoic acid)	0.03±0.00	0.02±0.00	nd
C18:1n9 (oleic acid)	17.58±0.59	54.41±0.76	62.84±2.05
C20:1 (11- eicosenoic acid)	0.27±0.03	0.02±0.00	nd
C22:1n9 (erucic acid)	nd	0.01±0.00	nd
C24:1n9 (nervonic acid)	nd	0.30±0.01	8.64±2.76
TMUFA	17.99±0.69	54.85±1.12	71.48±0.99
Polyunsaturated			
C18:2n6 (linoleic acid)	12.88±0.61	21.86±1.37	nd
C18:3n3 (α-linolenic acid)	54.29±1.08	0.44±0.04	7.80±0.84
C18:3n6 (γ-linolenic acid)	0.28±0.02	0.02±0.00	nd
C20:2 n6 (11,14eicosadienoic acid)	0.33±0.39	nd	nd
C20:3n3 (11,14,17-eicosatrienoic acid	0.08±0.00	nd	nd
TPUFA	67.83±0.91	22.47±1.12	7.80±0.84
Cis Fats	30.46±1.19	76.27±0.61	
Omega-3	54.38±1.08	0.54±0.17	7.80±0.84
Omega-6	13.20±0.57	21.88±1.37	nd
Omega-9	17.60±0.61	54.43±0.76	62.84±2.05
TPUFA:TSFA	4.81:1	0.99:1	0.39:1
n3PUFA:n6PUFA	4.13:1	0.02:1	---

TSFA: Total saturated fatty acids; TMUFA: total monounsaturated fatty acids; TPUFA: total polyunsaturated fatty acids; n3PUFA: omega-3 polyunsaturated fatty acids; n6PUFA: omega-6 polyunsaturated fatty acids. *Data on *X. caffra* previously published (8). nd: Not detected.

cell proliferation compared to the control. The growth of both Caco-2 and HEK-293 cells showed an overall trend of decreasing with an increasing seed oil concentration (Figures 1-3). *K. africana* seed oil significantly ($p<0.05$) suppressed Caco-2 and HEK-293 cell growth at all oil concentrations compared to the control (Figure 1). While there were no significant differences ($p>0.05$) in HEK-293 cell growth between the control and cells treated with *K. africana* seed oil at 20 mg/l, *K. africana* seed oil significantly ($p<0.05$) suppressed HEK-293 cell growth at higher doses and it caused greater growth suppression of Caco-2 cells compared to HEK-293 cells at all concentrations (Figure 1).

M. zeyheri seed oil significantly suppressed both Caco-2 and HEK-293 cell growth at concentrations greater or equal to 40 mg/l compared to control (Figure 2). The degree of suppression of growth for both cell lines by *M. zeyheri* seed oil was similar at all concentrations, except the 80 mg/l concentration, where suppression of growth was greater for HEK-293 than Caco-2 cells.

X. caffra seed oil significantly ($p<0.05$) suppressed Caco-2 and HEK-293 cell growth at all concentrations used compared to the control (Figure 3). There were no significant differences observed between Caco-2 and HEK-293 cell growth in any of the *X. caffra* seed oil concentrations, other than at 80 mg/l, where the *X. caffra* seed oil, unlike *K. africana* and M. zeyheri, significantly ($p<0.05$) suppressed growth of Caco-2 cells, more than HEK-293 cells (Figure 3).

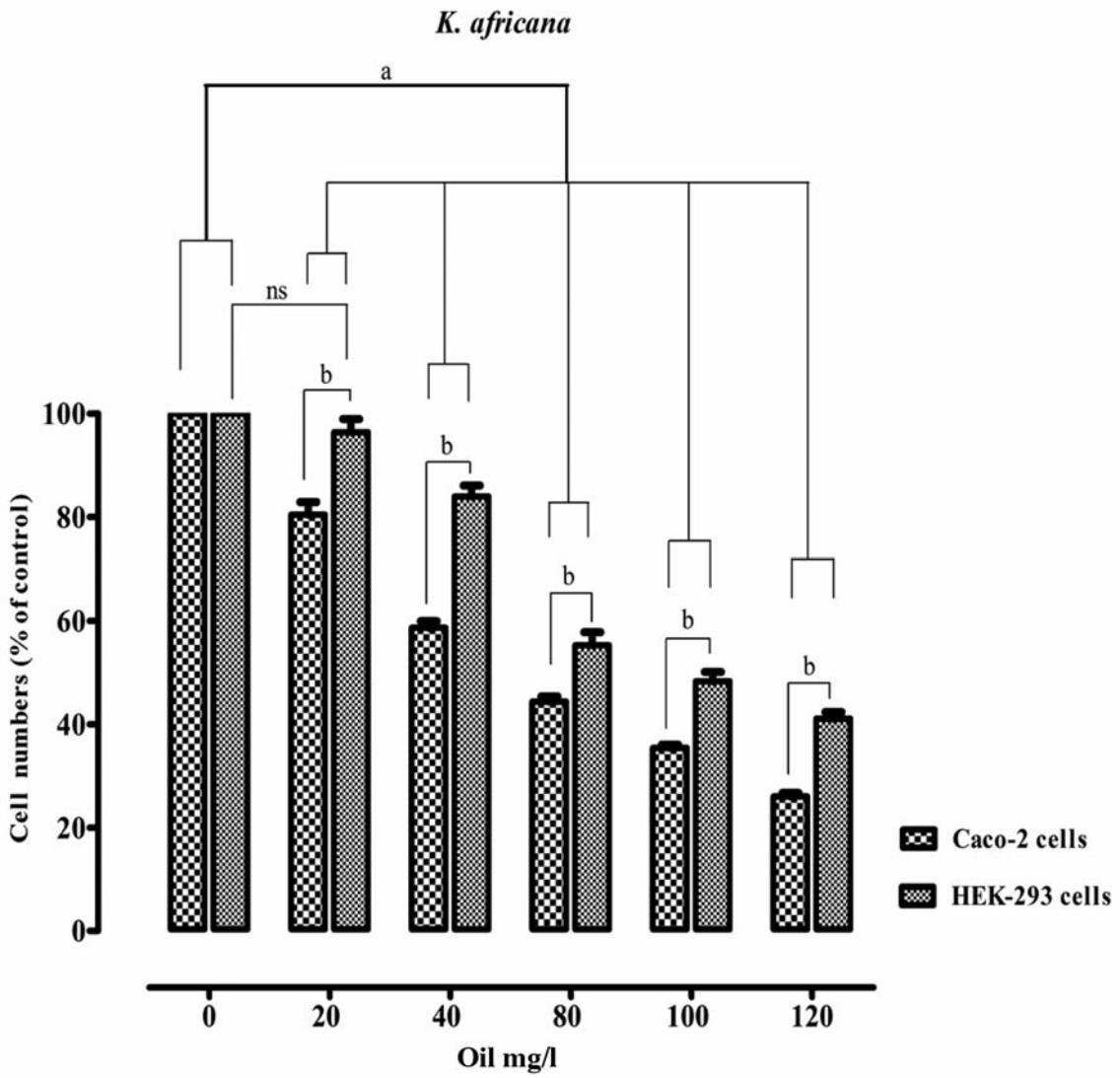

Figure 1. *Comparison of the effects of K. africana seed oil on Caco-2 and HEK-293 cells. 1×10^5 cells were plated per well and treated with the indicated oil concentrations. The cells were counted using the trypan blue dye exclusion method as described under materials and methods. Cell numbers (percent of control) for the Caco-2 and HEK-293 cells are shown for each oil concentration. The assays were performed in triplicate. [ns]Non-significant differences at $p>0.05$. [a]K. africana seed oil significantly ($p<0.05$) suppressed both Caco-2 and HEK-293 cell growth at all oil concentrations compared to control. [b]K. africana seed oil significantly ($p<0.05$) suppressed Caco-2 cell growth compared to HEK-293 cell growth at all oil concentrations.*

Discussion

The three tree seed oils consistently suppressed the growth of the two cell lines (Caco-2 and HEK-293) in a seemingly dose-dependent manner (Figures 1-3). In a similar study using shark liver oils, Davidson *et al*. (25) reported a trend towards growth inhibition of Caco-2 cells treated with liver oils from the great white and spinner shark species and attributed the trend to the n3 to n6 (n3:n6) polyunsaturated fatty acid ratio of the shark liver oils. The ratio of n3:n6 polyunsaturated fatty acids for *K. africana* seed oil (4.13) falls midway between the one observed for the great white (9.74) and the spinner (2.62) shark liver oils (25), while the ratio of *M. zeyheri* (0.02) is lower compared to the one observed in the shark liver oils. The observed Caco-2 cell growth suppression trend cannot be adequately explained by the n3:n6 ratio of the tree seed oils, since *M. zeyheri* seed oil with an n3:n6 polyunsaturated fatty acid ratio outside the observed ratios in shark liver oils, also resulted in a suppression of Caco-2 cell growth.

In animal models, fish oil, a rich source of eicosapentaenoic acid (EPA), an n3-polyunsaturated fatty acid, has been reported to reduce the induction of colorectal cancer by mechanisms

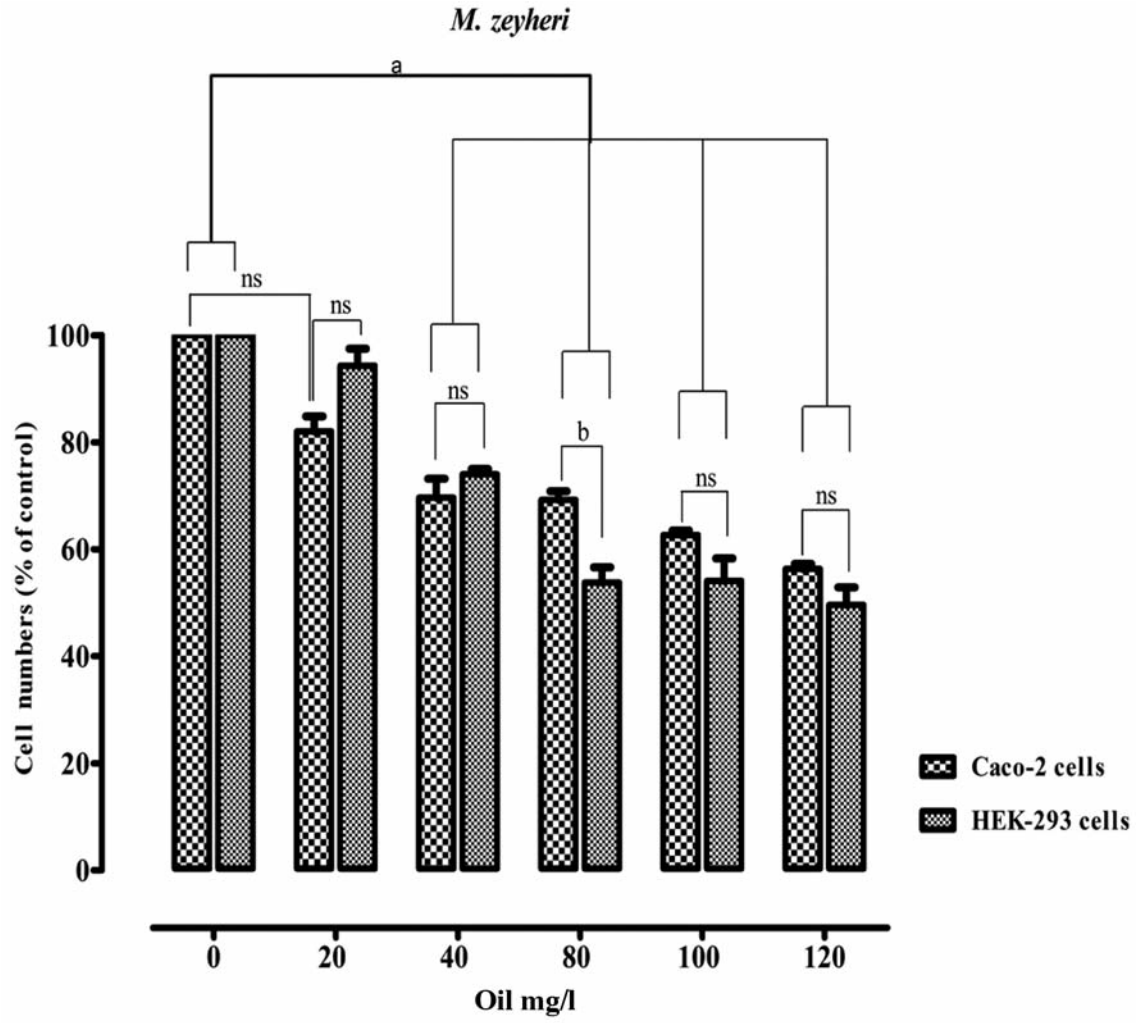

Figure 2. *Comparison of the effects of M. zeyheri seed oil on Caco-2 and HEK-293 cells. 1×10^5 cells were plated per well and treated with the indicated oil concentrations. The cells were counted using the trypan blue dye exclusion method as described under materials and methods. Cell numbers (percent of control) for the Caco-2 and HEK-293 cells are shown for each oil concentration. The assays were performed in triplicate. [ns]Non-significant differences with p>0.05. [a]M. zeyheri seed oil significantly (p<0.05) suppressed both Caco-2 and HEK-293 cell growth at oil concentrations ≥40 mg/l. [b]M. Zeyheri seed oil significantly (p<0.05) suppressed HEK-293 cell growth compared to Caco-2 cell growth at 80 mg/l oil concentration.*

that are thought to involve suppression of mitosis and increased apoptosis (26). In culture, EPA at 10 and 15 μg/ml concentrations reduced the growth rate of adherent HT29 cells, a human colon adenocarcinoma cell line (26). *K. africana*, *M. zeyheri* and *X. caffra* seed oils do not contain EPA but contain α-linolenic acid, an n3-polyunsaturated fatty acid that could have similar effects on the cells as EPA. In cells, α-linolenic acid is a substrate producing longer chains of more unsaturated n-3 fatty acids, including EPA and docosahexaenoic acid (27), thus the α-linolenic acid from the tree seed oils could have been metabolised to EPA (in the cells) which could then have led to suppressed Caco-2 and HEK-293 cell growth. Dommels *et al*. (28) reported growth inhibition of Caco-2 cells on incubation with linoleic acid and α-linolenic acid. The three tree seed oils contained α-linolenic acid; *K. africana* and *M. zeyheri* also contained linoleic acid (Table I). Although Dommels *et al*. (28) made use of purified linoleic and α-linolenic acid in their study which demonstrated growth inhibitory effects on Caco-2 cells, the presence (Table I) of linoleic and α-linolenic acid in large concentrations in the seed oils could account for the suppression of both Caco-2 and HEK-293 cell growth in a dose-dependent manner. The Caco-2 cells besides forming tight junctions (13, 14) and expressing structural properties similar to those of ileal enterocytes (15),

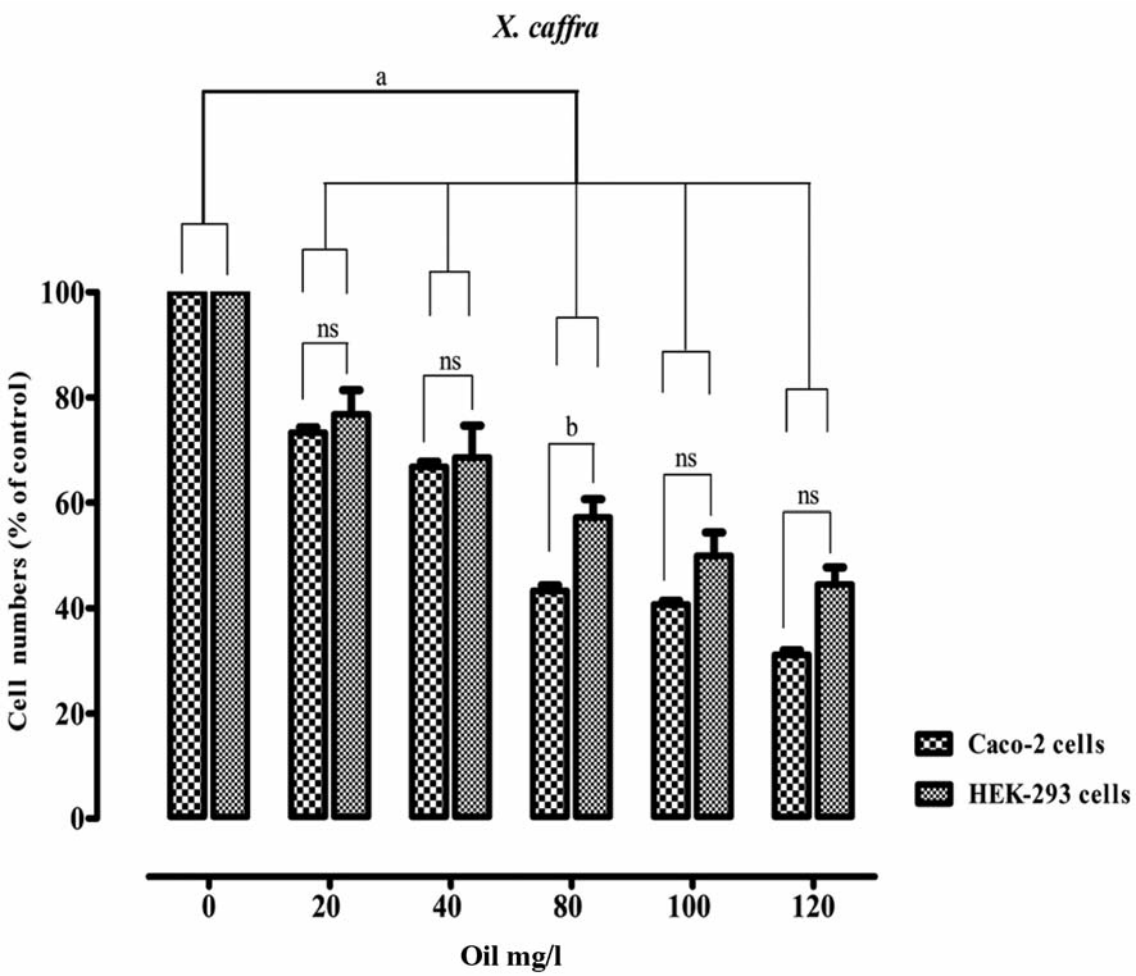

Figure 3. *Comparison of the effects of X. caffra seed oil on Caco-2 and HEK-293 cells 1×10^5 cells were plated per well and treated with the indicated oil concentrations. The cells were counted using the trypan blue dye exclusion method as described under materials and methods. Cell numbers (percent of control) for the Caco-2 and HEK-293 cells are shown for each oil concentration. The assays were performed in triplicate. [ns]Non-significant differences at p>0.05. [a]X. caffra seed oil significantly (p<0.05) suppressed both Caco-2 and HEK-293 cell growth at all oil concentrations compared to control. [b]X. caffra seed oil at 80 mg/l concentration significantly (p<0.05) suppressed Caco-2 cell growth compared to HEK-293 cell growth.*

they also express various levels of transporters on their cell membranes (29). In this study, the supressed Caco-2 cell growth in accordance with the increase in tree seed oil concentration (*K. africana*, *M. zeyheri* and *X. caffra* respectively) could have resulted from a possible alteration of the cells' membrane traits caused by the oils. Alteration of the cell membrane characteristics could lead to interference and/or partial blockage of channels that are responsible for nutrient uptake and excretion of metabolic waste by the cells, thus leading to subnormal levels of nutrient uptake and abnormal waste accumulation; conditions which might lead to growth suppression.

The trypan blue dye exclusion method, although being a rapid and easy method of measuring cell growth and viability, is not ideal. The method indirectly measures viability based on the cell membrane integrity, hence it is possible to have a cell whose viability has been compromised by its ability to grow and / or function, yet its membrane integrity can be transiently maintained. There is also the possibility that the cell membrane integrity could be seen as compromised (by letting the dye into the cell), but the method fails to cater for cells whose membranes (seemingly compromised) repair themselves and become viable. We want, however, to point out that our study focused on a preliminary determination of the potential effects of three tree seed oils on cell proliferation as a basis for further in-depth studies; hence the method sufficed for the objective set. In addition, as previously mentioned, there is the possibility that the oils altered the membrane structures, so the trypan blue dye exclusion method could be useful in detecting changes of membrane fluidity and permeability.

Conclusion

The suppression of cell proliferation by the tree seed oils strongly indicates antiproliferative effect of the *K. africana*, *M. zeyheri* and *X. caffra* seed oils on the two cell lines. Further work remains to be done, focusing on measuring DNA synthesis and metabolic profiles of treated cells in order to delineate the mechanisms responsible for growth suppression.

Acknowledgements

The Oppenheimer Memorial Trust, the University of the Witwatersrand's Faculty of Health Sciences Research Endowment Fund and the National Research Foundation are acknowledged for funding the study. The Molecular Physiology Laboratory, School of Physiology, University of the Witwatersrand is acknowledged for providing the laboratory space and equipment.

References

1 Owolabi OJ and Omogbai EKI: Analgesic and anti-inflammatory activities of the ethanolic stem bark extract of *Kigelia africana* (*Bignoiaceae*). Afr J Biotechnol *6*: 582-585, 2007.
2 Lemmens RHMJ: *Mimusops zeyheri* Sond. *In*: Prota 7: Timbers/Bios d'oeuvre. [CD-Rom] Louppe D (ed). pp. 89-94. Wageningen, Netherlands, Prota, 2005.
3 Lee RB: Mangongo: the ethnography of a major food resource. Ecol Food Nutr *2*: 307-321, 1973.
4 Janick J and Paull RE: *Mimusops zeyheri* Transvaal red milkwood. In: The Encyclopaedia of Fruit and Nuts. Janick J and Paull RE (ed). pp 831. Wallingford, UK: CABI, 2008.
5 Roodt V: Trees and shrubs of the Okavango delta, medicinal uses and nutritional value, The Shell Field Guide Series: Part 1. Shell Oil Botswana, Gaborone, Botswana, pp. 213, 1998.
6 Coates-Palgrave K: Trees of Southern Africa. (5th edn.) Struik Publishers, Cape Town, RSA, p. 34, 1988.
7 Chivandi E, Davidson BC and Erlwanger KH: A comparison of the lipid and fatty acid profiles from the kernels of the fruit (nuts) of *Ximenia caffra* and *Ricinodendron rautanenii* from Zimbabwe. Ind Crop Prod *27*: 29-32, 2008.
8 Chivandi E, Davidson BC, Pretorius B and Erlwanger KH: Proximate, mineral, amino acid, fatty acid, vitamin E, phytate phosphate and fibre composition of *Mimusops zeyheri* (Red Milkwood) seed. Int J Food Sci *46*: 555-560, 2011.
9 Chivandi E, Davidson BC and Erlwanger KH: *Kigelia africana* seed: proximate, mineral, vitamin E, fibre, amino acid and fatty acid composition. Int J Food Sci *46*: 2153-2158, 2011.
10 Goel G, Makkar HPS, Francis G and Becker K: Phorbol esters: structure, biological activity and toxicity in animals. Int J Toxicol *26*: 279-288, 2007.
11 Savanam CS and Bhaskara Rao VK: Cotton seed oil as a health oil. Pertanika J Trop Agric Sci *34*: 17-24, 2011.
12 Lai SM, Lim KW and Cheng HK: Margosa oil poisoning as a cause of toxic encephalopathy. Singapore Med J *31*: 463-465, 1990.
13 Rousset M: The human colon carcinoma cell lines HT-29 and Caco-2: two *in vitro* models for the study of intestinal differentiation. Biochimie *68*: 1035-1040, 1986.
14 Rousset M, Laburthe M, Pinto G, Chevalier G, Royer-Fessare E, Dussaulx E, Trugnan G, Borge N, Brun JL and Zweibaum A: Enterocyte differentiation and glucose utilization in the human colon tumor cell line Caco-2: modulation by forskolin. J Cell Physiol *123*: 377-385, 1985.
15 Vachon P and Beaulieu J: Transient mosaic patterns of pharmacological and functional differentiation in Caco-2 cell line. Gastroenterology *103*: 414-423, 1992.
16 Durocher Y, Perret S and Kamen A: High-level and high-throughput recombinant protein production by transient transfection of suspension-growing human 293-EBNA1 cells. Nucleic Acid Res *30*: E9, 2002.
17 Schlaeger E J and Christensen K: Transient gene expression in mammalian cells grown in serum-free suspension culture. Cytotechnology *30*: 71-83, 1999.
18 Government of Zimbabwe: Natural Regions and Farming Areas (Zimbabwe 1:1000000). Surveyor General, Harare, Zimbabwe: 2000.
19 Gwimbi P: Farmers' vulnerability to climate change in Gokwe District (Zimbabwe): impact and influencing factors. J Disaster Risk Studies *2*: 81-92, 2009.
20 Christopherson SW and Glass RL: Preparation of milk fat methyl esters by alcoholysis in an essential non-alcoholic solution. J Dairy Sci *52*: 1289-1290, 1969.
21 Bligh EG and Dyer WJ: A rapid method of total lipid extraction and purification. Canadian J Biochem Physiol *37*: 911-917, 1959.
22 Giangregorio A: The influence of fatty acids *in vitro* on mammalian cells from species differing in their fatty acyl desaturase capabilities. Ph.D. Thesis, University of the Witwatersrand, Johannesburg, South Africa, 1992.
23 Girao LAF: To determine *in vitro* effects of C18 fatty acids on normal, malignant and benign cell lines. Ph.D. Thesis, University of the Witwatersrand, Johannesburg, South Africa, 1988.
24 Paul J: Cell viability by trypan blue exclusion. *In*: Cell and Tissue Culture. Church Livingstone Press, London, UK, pp. 637-368, 1975.
25 Davidson BC, Rottanburg D, Prinz W and Cliff G: The influence of shark liver oils on normal and transformed cells in culture. In Vivo *21*: 333-338, 2007.
26 Clarke RG, Lund EK, Latham P, Pinder AC and Johnson IT: Effect of eicosapentaenoic acid on the proliferation and incidence of apoptosis in the colorectal cell line HT29. Lipids *34*: 187-1295, 1999.
27 Burdge GC: Metabolism of α-linolenic acid in humans. Prostagland, Leukotri Essent Fatty Acids *75*: 161-168, 2006.
28 Dommels YEM, Alink GM, Linssen JPH and van Ommen B: Effects of n-6 and n-3 polyunsaturated fatty acids on gap junctional intercellular communication during spontaneous differentiation of the human colon adenocarcinoma cell line Caco-2. Nutr Cancer *42*: 125-130, 2002.
29 Mandagere A, Thompson T and Hwang K: Graphical model for estimating oral bioavailability of drugs in humans and other species from Caco-2 cell permeability and *in vitro* liver enzyme metabolic stability rates. J Med Chem *45*: 304-311, 2002.

Received September 16, 2011
Revised November 10, 2011
Accepted November 11, 2011