South African Journal for Science and Technology
ISSN: (Online) 2222-4173, (Print) 0254-3486

Effects of extracts of *Sutherlandia frutescens* on drug transport and drug metabolising enzymes

Author:
David R Katerere

Affiliations:
Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, South Africa

Corresponding author:
David Katerere
Kateredr@tut.ac.za

Dates:
Received: 17/10/2017
Accepted: 24/08/208
Published:

How to cite this article:
David R Katerere, Effects of extracts of *Sutherlandia frutescens* on drug transport and drug metabolising enzymes, *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie* 37(1) (2018)

'n Afrikaanse vertaling van die manuskrip is aanlyn beskikbaar by http://www.satnt.ac.za/index.php/satnt/article/view/663

Drug-drug and drug-herb interactions must be considered in both pre-clinical studies and clinical use. This study set out to investigate the possible interactions caused by extracts of *Sutherlandia frutescens* (Sutherlandia) at drug transporter (P-gp) level and at hepatic metabolic enzyme level (CYP 450).

MDCK-MDR1 cells were treated with aqueous Sutherlandia extract (6.8 mg/ml to 1.7 µg/ml) in the presence of amprenavir, a protease inhibitor used to treat HIV infection. For CYP inhibition the P450-Glo™ assay kit was used and aqueous and organic extracts of Sutherlandia tested. Higher doses of Sutherlandia extract from 850 µg/ml upwards adversely affected tight junctions (TJ) as shown by the reduced transepithelial electric resistance (TEER) readings. At lower concentrations, Sutherlandia caused increased amprenavir permeability in a dose-dependent manner, suggesting that the extract inhibited P-gp and reduced amprenavir efflux. In the CYP inhibition assays, Sutherlandia extracts showed IC_{50} values above 10 µg/ml for all enzyme isoforms, hence CYP enzyme inhibitory interactions at this level are not expected to be clinically significant.

Keywords: herb-drug interactions, traditional medicine, antiretroviral, permeability, CYP inhibition

Die invloed van ekstrakte van *Sutherlandia frutescence* op die vervoer en metabolisme van ander geneesmiddels: Interaksies tussen geneesmiddels en interaksies met kruiemedisyne moet in ag geneem word tydens pre-kliniese studies en in kliniese gebruik. In hierdie studie is moontlike interaksies tussen *Sutherlandia frutescence* (Sutherlandia) ekstrakte en medisyne op die vlak van oordraers (P-gp) en lewer metaboliese ensieme (CYP 450) ondersoek.

MDCK-MDRI selle is behandel met 'n waterekstrak van Sutherlandia (1.7 µg/ml tot 6.8 mg/ml) in die teenwoordigheid van amprenavir, 'n protease inhibeerder wat gebruik word om MIV infeksies te behandel. Vir die CYP inhibering is die P450-Glo™ essajeerstel gebruik met waterige en organiese ekstrakte van Sutherlandia. Dosisse hoër as 850 µg/ml het die hegte koppelings (HK) beïnvloed soos blyk uit die verlaagde transepiteel elektriese weerstand (TEEW). By laer konsentrasies is die amprenavir permeabiliteit in 'n dosis-verwante verhouding verhoog. Dit mag veroorsaak wees deurdat dit die ekstrak P-gp inhibeer het, met gevolglike laer uitpomp van amprenavir. Die IC_{50} waardes in die CYP inhiberingstudies was > 10 ug/ml vir al die ensiemisovorme. Gevolglik is die CYP inhibering waarskynlik nie klinies betekenisvol nie.

Sleutelwoorde: kruiemedisyme-geneesmiddel-interaksies, tradisionele medisyne, antiretroviraal, permeabiliteit, CYP

Introduction

The interactions of drugs with other drugs, chemicals and herbs to which a patient might be exposed are important considerations in pre-clinical drug discovery studies and clinical use. These interactions may result in either sub-therapeutic or toxic concentrations of the victim drug (i.e. the drug which is negatively affected by drug-drug interactions) being absorbed into the bloodstream. Interactions occur primarily at luminal transporter level [e.g. Permeability glycoprotein (P-gp)], or by inhibiting or inducing the drug metabolising enzymes (DME) of which the cytochrome P450 complex is the most important (Al Omari & Murry 2007).

Most of the studies done on drug-herb interactions are concerned with phytomedicines which are popular in industrialied countries, for example St John's wort (SJW) and ginseng (Murphy, Kern *et al.* 2005; Lee, Lee *et al.* 2010), but little attention has been paid to herbs which are used in Africa and Asia where traditional medicine remains an important part of health care (WHO 2002; Okigbo, Eme *et al*. 2008). This study set out to investigate the possible drug-herb

interactions caused by Sutherlandia, a popular African herb, at transporter level (P-gp) and at DME level (CYP 450).

The increased use of complementary and alternative medicine (CAM) and herbal supplements is well-documented. In particular it has been recognised that there is a high tendency to co-medicate with CAM by persons taking highly active anti-retroviral therapy (HAART) (Bica, Tang *et al.* 2003; Monera, Wolfe *et al.* 2008). This places such patients at high risk for drug-herb interactions which may result in treatment failure (and the possible emergence of resistant retroviral strains) or adverse drug reaction (ADR) (and possible non-compliance) (Elvin-Lewis 2001). Such interactions have been documented when patients are concurrently using anti-coagulants, anti-depressants and anti-epileptics (Zhou, Zhou *et al.* 2007; Harish Chandra & Veeresham 2011).

Sutherlandia is a popular botanical derived from the ground leaves of *Sutherlandia frutescens* (L.) R.BR. [syn. *Lessertia frutescens* (L.) Goldblatt and J.C. Manning]. Commonly known as cancer-bush (unwele in Zulu) this plant species is endemic to the karoo biome of southern Africa and has a long history of traditional use for stress disorders, cancer, diabetes and dysentery (Prevoo, Swart *et al.* 2008; van Wyk & Albrecht 2008). In the past decade it has been popularised as a so-called "adaptogen" in stress management (Prevoo, Swart *et al.* 2008) and an "immune booster" for counteracting cachexia in HIV and AIDS (van Wyk & Albrecht 2008). Clinical trials to establish efficacy in HIV and AIDS treatment have been ongoing for the past few years and results are yet to be published.

Of relevance to the present study is the fact that Sutherlandia is often taken by patients on HAART or cancer chemotherapy. Persons using both treatments are already prone to ADR and drug-drug interactions because of polypharmacy (Piscitelli & Gallicano 2001; Neuman, Monteiro *et al.* 2006). The use of traditional medicine and herbal supplements, which is widespread in southern Africa, complicates therapy even further.

This study set out to investigate the effects of Sutherlandia on the permeability of amprenavir using the Madin Darby canine kidney (MDCK) cell line transfected with P-gp (MDR-1) transporters, and also on CYP450 inhibition.

Permeability of a drug and how it may be affected by other drugs, herbs, food and disease states is an important consideration in understanding drug absorption, which in turn will influence efficacy and/or toxicity (Volpe 2008). CYP450 inhibition leads to increased bioavailability of the victim drugs, which may lead to ADRs. In this case we were interested in establishing potential herb-drug interactions on the most important human drug metabolising enzymes.

Materials and methods

MDCK permeability assay

Materials

Dulbecco's Modification of Eagle's Medium (DMEM) with GlutaMAX (GIBCO); Penicillin-Streptomycin (Pen/Strep, Sigma); Trypsin-EDTA (0.25% trypsin GIBCO); Hanks' Balanced Salt Solution (HBSS, Sigma); HEPES Buffer (Sigma); Fetal Bovine Serum (FBS, GIBCO); GF120918 [MDR1 inhibitor was obtained from GSK (RTP, NC)]; 12-well Transwell® (Corning Costar Cooperation USA); HPLC vials (Agilent); 500 ml Filter System (Fisher); transepithelial electrical resistance (TEER) reader; CO_2 shaker incubator; Deep-well plates (96 DWP) or vials; LC-MS plates/vials; (Madin Darby canine kidney cells transfected with the human MDR1 gene) (Dr. Piet Borst, NKI-AVL, Amsterdam, The Netherlands); leaf powder of *Sutherlandia frutescens* (purchased from Parceval, Cape Town, South Africa).

Seeding of transwell plates

The culturing of MDCK-MDR1 cells was done using standard methods in T-75 flasks three days prior to seeding. The cells were then trypsinised and resuspended in 9 ml of cell culture medium, thoroughly mixed by aspiration and then counted using a haemocytometer and diluted with sufficient medium to achieve a density of 330 000 cells per well in 0.5 ml medium. Medium only (1.5 ml) was aliquoted into the basolateral chambers of the Transwells® with 0.5 ml of the diluted cell suspension being aliquoted into the apical chamber. The seeded transwells were incubated overnight and then media changed thereafter. After a further 48 hours the cells were judged to be ready for use in the permeability assay.

The permeability assay

For the permeability assay, the cell culture medium was removed from Transwells® and replaced with Transport Medium (TM) with organic Sutherlandia extract at concentrations ranging from 6.8 mg/ml to 1.7 µg/ml (0.5 ml apical, 1.5 ml basolateral). One set of Transwells® contained TM without P-gp inhibitor (-) and the other had the standard inhibitor GF120918 (+). Each Transwell® was labelled appropriately and incubated for 30–60 minutes. During this time, dosing preparations were made. Sutherlandia extract was serially diluted to concentrations from 6.8 mg/ml to 1.7 µg/ml in DMSO. Two sets of dosing solutions were made – one without inhibitor was made with 6 µl of amprenavir, 34 µl of extract and 2 ml of TM and the other with inhibitor was made with amprenavir (6 µl), GF120918 (2 µl), DMSO (32 µl) and 2 ml of TM.

Following the pre-incubation, TEER values were measured and recorded for each Transwell®. The contents of both the donor and receiver chambers were aspirated and

1.5 ml TM with/without GF120918 was added to each basolateral compartment. The dosing solutions (0.4 ml) (with and without GF120918) were added to each apical compartment.

The Transwells® were then incubated, with shaking at 160 rpm, in a CO_2 incubator at 37 ° for 60 minutes after which the donor chambers were removed from the plates and the TEER values were again recorded for each well. Aliquots of 200 µL of donor and receiver samples were taken separately and diluted with 200 µL of MeOH in deep well plates. The plates were refrigerated for 30 min and then mixed five times with a pipette. Aliquots of donor (20 µL) and receiver (80 µL) were then placed separately into 96 well LC-MS plates and diluted to 200 µL with 50/50 TM/MeOH.

To check for recovery and the integrity of the transmembrane, fresh TM was loaded into the apical (0.5 ml) and basal (1.5 ml) chambers and the transwells once again incubated with shaking at 160 rpm, in a CO_2 incubator at 37 ° for 60 minutes. Thereafter, further TEER readings were measured.

LC-MS method

All samples were assayed for amprenavir concentrations by LC-MS/MS using electrospray ionisation. Analyte (10 uL) was injected onto an Aquasil C18 DASH HTS 20 × 2.1 mm column (Thermo Scientific) and eluted by gradient of aqueous (A) (5 mM ammonium acetate + 0.1% formic acid in water) and organic (B) (5 mM ammonium acetate + 0.1% formic acid in methanol) at 600 uL/min (90% A/10% B to 5% A/95% B) for 1.5 min. Ion transitions (m/z 506.2 > 156.0) were detected by multiple reaction monitoring (MRM) in positive ion mode by API 4000 QTRAP (Applied Biosystems) mass spectrometer. The ionspray voltage was set at 5000v, temperature at 500 °C, curtain gas at 10, dwell time 100 ms, Declustering Potential 81 v, collision energy (CE) 26 v, cell exit potential (CXP) 16 v.

CYP inhibition assay

Materials

White 96 well plates (Costar® Cat # 3912), all luminescent reagents were purchased as a P450-Glo™ kit from Promega (Madison, WI, USA) (luciferin-free water, recombinant human CYP enzyme membranes, NADPH regenerating solution luciferin detection reagent), standard inhibitors were obtained from Sigma-Aldrich [α-naphthoflavone (for 1A2), sulfaphenazole (2C9), troglitazone (2C19), sertraline (2B6) and ketoconazole (3A4)].

Luminescence assay

The P450-Glo™ assay kit from Promega (Madison, WI, USA) was used. In brief, a reaction mixture of luciferin-free water, phosphate buffer and the individual CYP enzyme membranes with their specific pro-luciferin substrates were mixed and placed in white 96 well plates (Costar® Cat # 3912). The treatment was performed in triplicate and serially diluted. Three controls were used *viz.* one with control membranes, one set with vehicle and the third with appropriate standard inhibitors. Aqueous and organic (1:1 dichlomethane:methanol) extracts of Sutherlandia powder were made up into a stock solution to give final serial dilutions from 100 to 1 µg/ml. The plate was pre-incubated for 10 min at 37 °C and then NADPH regenerating solution was added and the plate incubated again at 37 °C for varying periods depending on the enzyme (as specified in the Promega protocol). Thereafter luciferin detection reagent was added and the plate incubated at room temperature for the specified time and then luminescent activity was read using the Wallac® luminometer with Envision® software (Perkin Elmer, Mass, USA). The data was processed by MS Excel and then transformed by GraphPad to give IC_{50} readings.

TABLE 1: TEER values and apparent permeability (Papp) of MDCK-MDR1 cells treated with various concentrations of dichloromethane – extracted Sutherlandia.

Name of extract	Conc (mg/ml)	TEER before incubation (mv)	TEER after incubation (mv)	TEER 1 hour after incubation (mv)	Papp (nm /s) *
Suther 001	6.8000	24.7 (2.1)	11.7 (3.3)	6.7 (0.9)	724
Suther 002	3.4000	50.3 (4.9)	26.3 (4.1)	8.0 (0.8)	596
Suther 003	1.7000	58.7 (6.8)	44.0 (8.8)	4.3 (0.5)	428
Suther 004	0.8500	61.3 (0.9)	39.0 (5.4)	3.3 (0.5)	487
Suther 005	0.4250	63.7 (3.3)	61.3 (4.7)	23.3 (2.9)	343
Suther 006	0.2125	53.0 (6.4)	56.0 (7.9)	38.3 (4.6)	242
Suther 007	0.1063	61.0 (1.6)	70.0 (5.0)	46.0 (1.4)	116
Suther 008	0.0531	53.3 (5.2)	63.3 (9.4)	45.7 (4.5)	87
Suther 009	0.0266	58.0 (1.6)	67.3 (5.3)	52.3 (2.5)	38
Suther 010	0.0133	58.7 (1.2)	64.0 (5.0)	55.3 (2.6)	35
Suther 011	0.0066	59.0 (0.8)	67.7 (7.4)	58.7 (1.2)	21
Suther 012	0.0033	57.3 (1.7)	62.7 (7.1)	53.7 (2.1)	20
Suther 013	0.0017	60.0 (0.8)	66.7 (2.5)	53.3 (0.9)	16
GF120918	0.0000	55.0 (2.2)	68.3 (2.4)	51.0 (5.7)	479
N	2.0000	52.3 (3.4)	58.3 (6.9)	47.7 (1.9)	76

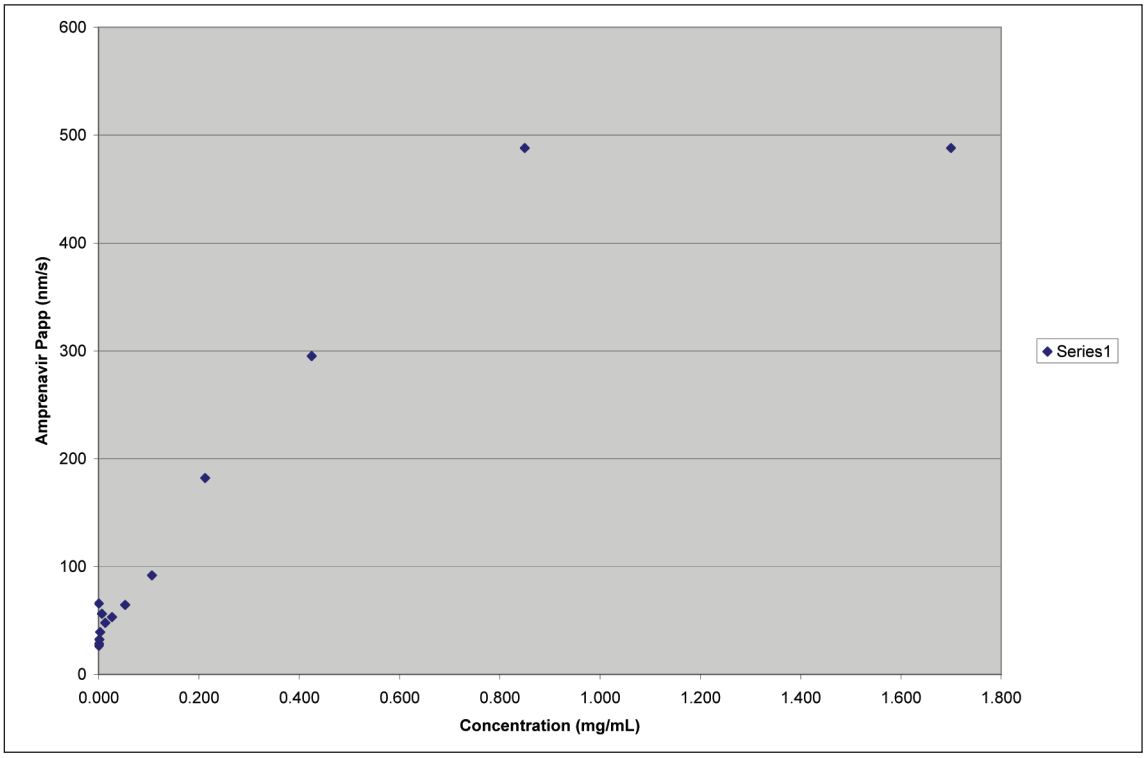

FIGURE 1: Graph showing the concentration-dependent relationship between Sutherlandia concentration and apparent permeability of amprenavir

Results

The CYP inhibition studies investigated both aqueous and organic extracts of Sutherlandia. Only organic extracts were used for the transporter studies.

Permeability assay

The TEER readings before incubation, immediately after incubation and one hour after incubation as well as the apparent permeability (Papp) are shown in Table I. TEER measures the integrity of the tight junctions (TJ) of cells and the normal readings for MDCK range from 55 to 70 millivolts. TJ are continuous, circumferential seals around cells that serve as a physical barrier and aid in the maintenance of cell polarity (Furuse, Fujita *et al*. 1998).

In the present study, the higher doses of Sutherlandia extract from 850 µg/ml upwards adversely affected TJ as shown by the reduced TEER readings. However, lower concentrations, 850 µg/ml to 53 µg/ml, seem to cause irreversible damage as the post-treatment readings suggest. This may imply that the extract is toxic to MDCK cells in general or specifically targets tight junction peptides e.g. occludins and claudins (Gonzalez-Mariscal, Nava *et al.* 2005). Minocha *et al.* (Minocha, Mandava *et al.* 2011) showed that ethanol extracts of Sutherlandia showed no cytotoxic effects at concentrations up to 1 400 µg/ml in LS-180 cells .

Amprenavir permeability increased in a dose-dependent manner i.e. permeability increases with increasing Sutherlandia concentration from 53 µg /ml. (Figure 1). This seems to suggest that the extract is inhibiting P-gp and reducing amprenavir efflux.

Mills *et al.* (Mills, Foster *et al.* 2005), using an enzyme kit, reported that a 100 mg/ml aqueous Sutherlandia extract inhibited P-gp by 31% compared to the control, verapamil while Brown *et al.* (Brown, Heyneke *et al.* 2008) reported that water decoctions of Sutherlandia showed low inhibition of P-gp in Caco-2 cells with nevirapine as substrate. This study seems to suggest that the organic extract is a more potent P-gp inhibitor and since Sutherlandia is consumed as whole leaf tablets, this is a significant finding which has not been previously reported.

CYP inhibition assays

In the CYP inhibition assays, the IC_{50} values for Sutherlandia extracts were above 10 µg/ml for all enzyme isoforms which implies a diminished potential for drug interactions at the tested concentrations.

General discussion

The practical implications of this study may be that persons self-medicating with Sutherlandia may be prone to intoxication if they are also co-medicating with drugs which are P-gp substrates, e.g. HIV protease inhibitors, digoxin, cyclosporine, macrolide antibiotics, anti-neoplastics and verapamil (Lee & Gottesman 1998; Huisman, Smit *et al.* 2002; Bauer, Hartz *et al.* 2005; Mills, Foster *et al.* 2005). This may be particularly important where such drugs have a narrow therapeutic index. The effects of organic extracts of Sutherlandia on P-gp activity are important because

Sutherlandia is not only consumed as a tea (i.e. aqueous decoction) but also in a tablet form or tincture. The latter dosage forms contain components which fractionate into both aqueous and organic solvents.

The effect of Sutherlandia extracts on tight junctions is noteworthy as it may increase passive paracellular diffusion. TJ are known to regulate paracellular transiting of hydrophilic xenobiotics (Gonzalez-Mariscal, Nava *et al.* 2005). The result of both P-gp inhibition and TJ opening is potential drug intoxication. In practical terms this may necessitate the adjustment of dosages of drugs in populations where the use of herbs such as Sutherlandia is prevalent. Alternatively, the use of such herbal products should be highly restricted and monitored, in contrast to the current situation where they are widely available and actively promoted in HIV and AIDS patients. Both suggestions will be complicated to implement and in any case further studies probably need to be conducted.

Both aqueous and organic extracts of Sutherlandia showed no potential for inhibiting CYP enzymes. A previous study however shows that co-administration of Sutherlandia with nevirapine for five days caused a 50% decrease in important pharmacokinetic parameters (i.e $AUC_{0\text{-inf,}}$ C_{max} and t_{max}) in rats (Minocha, Mandava *et al.* 2011). In addition, a significant increase in hepatic and intestinal mRNA expression of CYP3A2 was found implying that Sutherlandia was inducing CYP3A2 enzymes. At very high concentrations (> 600 ug/ml) Sutherlandia inhibited CYP3A2 enzymes. These findings are important because they were carried out on live animals and cell cultures. However, an ethanol fraction reconstituted in water was used which may explain the differences with our study. As alluded to earlier, Sutherlandia is commonly taken as a whole leaf extract, therefore it is worth using different extracts to get a complete picture of its interactions.

Acknowledgements

This work was done as part of the NRF/Emory University/ Scynexis Advanced Drug Discovery Programme for which the author was a beneficiary. The author wishes to thank Dr Liotta (Emory University) and Dr Yves Reibel (Synexis Inc) for hosting him.

References

Al Omari A and Murry D. (2007). Pharmacogenetics of the Cytochrome P450 Enzyme System: review of current knowledge and clinical significance. *Journal of Pharmacy Practice* 20 207-218.

Bauer B, Hartz AM, Fricker G and Miller DS. (2005). Modulation of p-glycoprotein transport function at the blood-brain barrier. *Experimental Biology and Medicine* 230(2): 118-127.

Bica I, Tang AM, Skinner S, Spiegelman D, Knox T, Gorbach S, et al. (2003). Use of complementary and alternative therapies by patients with human immunodeficiency virus disease in the era of highly active antiretroviral therapy. *Journal of Alternative and Complementary Medicine* 9(1): 65-76.

Brown L, Heyneke O, Brown D, van Wyk JP and Hamman JH. (2008). Impact of traditional medicinal plant extracts on antiretroviral drug absorption. *Journal of Ethnopharmacology* 119(3): 588-592.

Elvin-Lewis M. (2001). Should we be concerned about herbal remedies. *Journal of Ethnopharmacology* 75(2-3): 141-164.

Furuse M, Fujita K, Hiiragi T, Fujimoto K and Tsukita S. (1998). Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *The Journal of Cell Biology* 141(7): 1539-1550.

Gonzalez-Mariscal L, Nava P and Hernandez S. (2005). Critical role of tight junctions in drug delivery across epithelial and endothelial cell layers. *The Journal of Membrane Biology* 207(2): 55-68.

Harish Chandra R and Veeresham C. (2011). Herb-drug interaction of noni juice and Ginkgo biloba with phenytoin. *Pharmacognosy Journal* 2(18): 33-41.

Huisman M, Smit J, Crommentuyn K, Zelcer N, Wiltshire H, Beijnen J, et al. (2002). Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. AIDS 16: 2295-2301.

Lee CG and Gottesman MM. (1998). HIV-1 protease inhibitors and the MDR1 multidrug transporter. *The Journal of Clinical Investigation* 101(2): 287-288.

Lee YH, Lee BK, Choi YJ, Yoon IK, Chang BC and Gwak HS. (2010). Interaction between warfarin and Korean red ginseng in patients with cardiac valve replacement. *International Journal of Cardiology* 145(2): 275-276.

Mills E, Foster BC, van Heeswijk R, Phillips E, Wilson K, Leonard B, et al. (2005). Impact of African herbal medicines on antiretroviral metabolism. *AIDS* 19(1): 95-97.

Minocha M, Mandava NK, Kwatra D, Pal D, Folk WR, Earla R, et al. (2011). Effect of short term and chronic administration of Sutherlandia frutescens on pharmacokinetics of nevirapine in rats. *International Journal of Pharmaceutics* 413(1–2): 44-50.

Monera TG, Wolfe AR, Maponga CC, Benet LZ and Guglielmo J. (2008). Moringa oleifera leaf extracts inhibit 6beta-hydroxylation of testosterone by CYP3A4. *Journal of Infection in Developing Countries* 2(5): 379-383.

Murphy PA, Kern SE, Stanczyk FZ and Westhoff CL. (2005). Interaction of St. John's Wort with oral contraceptives: effects on the pharmacokinetics of norethindrone and ethinyl estradiol, ovarian activity and breakthrough bleeding. *Contraception* 71(6): 402-408.

Neuman MG, Monteiro M and Rehm J (2006). Drug interactions between psychoactive substances and antiretroviral therapy in individuals infected with human immunodeficiency and hepatitis viruses. *Substance Use and Misuse* 41(10-12): 1395-1463.

Okigbo R, Eme U and Ogbogu S. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnology and Molecular Biology Reviews* 3 (127-134).

Piscitelli SC and Gallicano KD. (2001). Interactions among drugs for HIV and opportunistic infections. *The New England Journal of Medicine* 344(13): 984-996.

Prevoo D, Swart P and Swart AC. (2008). The influence of Sutherlandia frutescens on adrenal steroidogenic cytochrome P450 enzymes. *Journal of Ethnopharmacology* 118(1): 118-126.

van Wyk BE and Albrecht C. (2008). A review of the taxonomy, ethnobotany, chemistry and pharmacology of Sutherlandia frutescens (Fabaceae). *Journal of Ethnopharmacology* 119(3): 620-629.

Volpe DA. (2008). Variability in Caco-2 and MDCK cell-based intestinal permeability assays. *Journal of Pharmaceutical Sciences* 97(2): 712-725.

WHO (2002). Evaluation of certain mycotoxins 906.

Zhou S-F, Zhou Z-W, Li C-G, Chen X, Yu X, Xue CC et al. (2007). Identification of drugs that interact with herbs in drug development. *Drug Discovery Today* 2(15–16): 664-673.