



Pharmacokinetic interactions: The effects of selected herbal extracts on permeation of P-glycoprotein substrate drugs across excised pig intestinal tissue

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ABSTRACT

Introduction: There is growing concern that co-administered herbal medicines may alter pharmacokinetics and, therefore, the efficacy and toxicity of Western drugs. The aim of this study was to investigate the potential membrane permeation modulating effects of four herbal extracts, i.e., *Harpagophytum procumbens*, *Hoodia gordonii*, *Leonotis leonurus*, and *Vitis vinifera* on a model compound, Rhodamine 123 (RH-123).

Methods: An *in vitro* permeation model, i.e., excised pig intestinal tissue, mounted to test chambers in a Sweetana-Grass diffusion apparatus, was used to measure the bi-directional transport of RH-123 in the presence and in the absence of four herbal extracts. The concentration of transported RH-123 in each sample was determined by means of fluorescence spectroscopic analysis. The integrity of the mounted jejunum tissue during experimentation was confirmed by measuring the permeation of Lucifer Yellow through these membranes. Trans-epithelial electrical resistance (TEER) of the mounted membranes was also measured at the onset and termination of each experiment to monitor whether tight junction modulation occurred.

Results: *H. procumbens* extract increased the secretory transport of RH-123, indicative of the induction of P-glycoprotein (P-gp) mediated efflux. *H. gordonii* extract also increased RH-123's absorptive transport, coupled with a subsequent decrease in its secretory transport, indicating the P-gp related efflux inhibition. Contrary, *L. leonurus* extract reduced RH-123's absorptive transport, accompanied by an increase in its secretory transport. *V. vinifera* seed extract, however, increased both the absorptive and secretory transport of RH-123. A reduction in TEER was observed in the presence of *V. vinifera* extract, indicating the modulation of tight junction integrity.

Conclusion: The *ex vivo* pharmacokinetics interactions recorded in the current study suggest that the co-administration of herbal medicines could alter the extent of membrane permeation of Western drugs.

Implication for health policy/practice/research/medical education:

The co-administration of selected herbal medicines exhibited *ex vivo* pharmacokinetic interactions, which could alter the extent of membrane permeation of Western drugs. This may have significant implications on drug treatment regimes.

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Introduction

Many patients use herbal health products as an alternative to, or in combination with prescribed medicines, due to factors such as mounting scientific recognition of such products for the prevention and treatment of diseases, a general trend towards self-medication, dissatisfaction

with the high costs and the outcomes of allopathic medicine regimes and the improved quality of herbal medicinal products in recent years (1-3). However, undesirable interactions between herbal products and pharmaceutical drugs may result from pharmacokinetic or pharmacodynamic mechanisms (4-6).

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Harpagophytum procumbens (Burch.) DC. ex Meisn. is a medicinal plant that exhibits anti-inflammatory, analgesic, antioxidant, antidiabetic, antimicrobial, antimalarial, and anticancer activities (7-9). It is used for the treatment of conditions such as dyspepsia, wounds and skin rashes, for stimulating the appetite, blood diseases, fever, urinary tract infections, postpartum pain, sores, ulcers, boils as well as liver and kidney disorders (9-11). No negative interactions have been reported regarding the concomitant use of *H. procumbens* and conventional medicines for the treatment of rheumatoid arthritis (12). However, due to its cardiac activity, chronic treatment with *H. procumbens* is discouraged (13). Commercial preparations of *H. procumbens* have shown to inhibit P-glycoprotein (P-gp) activity (14), while extracts of *H. procumbens* can modulate P-gp expression and P-gp activity (14,15). Furthermore, *H. procumbens* was shown to slightly inhibit some CYP450 enzymes (16), suggesting that it may cause herb-drug interactions.

Hoodia gordonii (Masson) Sweet ex Decne has anti-obesity potential that is believed to suppress appetite and enhance anorexic action (17).

Leonotis leonurus (L.) R.Br. (wild dagga) is used to treat multiple illnesses, both internally and externally (18,19). Traditionally, a decoction is prepared to relieve coughs, colds, and bronchitis. The Khoisan (an indigenous tribe in South Africa) people also utilize such decoctions to treat constipation and nausea. The aerial parts (leaves and flowers) are often used for the treatment of chest infections, flu, intestinal worms, dysentery, influenza, constipation, and headaches (20,21). The extracts of *L. leonurus* that contain the diterpenoid marrubiin, were shown to restrain the hypercoagulable and inflammatory states, often associated with diabetes and cardiac conditions, such as hypertension, and is thus known to alleviate symptoms associated with these conditions (22,23).

Vitis vinifera (L.) seed extract, derived from the seeds of grapes, contains procyanidins that are used to treat conditions such as blood clotting, cancer, nausea, inflammation, cholera, smallpox, eye infections, as well as skin, kidney, and liver diseases. Procyanidins limit platelet adhesion, which in turn mediates anticoagulant effects. It has been reported that *V. vinifera* seed extract has anti-ulcer activity on stomach lesions, which can also be attributed to the presence of the procyanidin oligomers (24,25).

Modulation of adenosine triphosphate (ATP)-binding cassette (ABC) transporters, such as P-gp, can readily occur in the presence of exogenous compounds, such as herbal medicines, foods, beverages, and Western drugs (26). Toxic blood concentrations of the co-administered drug may develop in some cases of transporter inhibition, due to limited drug efflux. Conversely, drug treatment failure may occur upon induction of P-gp related efflux transporters, resulting in sub-therapeutic plasma levels of the drug (26). The aim of this study was to investigate

the potential membrane permeation modulating effects of four herbal extracts, *H. procumbens*, *H. gordonii*, *L. leonurus*, and *V. vinifera* on a model compound, Rhodamine 123 (RH-123).

Materials and Methods

Preparation of the herbal extracts

Hoodia gordonii, *H. procumbens* and *V. vinifera* (seed) herbal extracts were purchased from Parceval Pharmaceuticals (Cape Town, South Africa). Dried *L. leonurus* plant leaf material was donated by professor Alvaro Viljoen (Tshwane University of Technology, Pretoria). Verapamil was donated by Novartis (South Africa). Excised pig intestinal jejunum tissue was collected from the local abattoir (Potchefstroom, South Africa).

Extracts of *H. procumbens*, *H. gordonii* and *V. vinifera* (seed) were purchased in liquid form (ethanol-based infusions). Ethanol was removed from these solutions under reduced pressure through evaporation (Rotavapor® R-100) at a temperature of 40°C. The remaining solid fraction was diluted with distilled water and frozen in ice trays at -80°C. Two extracts were prepared from the *L. leonurus* leaf material. An ethanol-based extract was prepared by adding 100 g of leaf material to 150 mL of ethanol, while the second extract was prepared by adding the same amount of leaf material to 150 mL of distilled water. These mixtures were agitated for a period of 48 hours, using a mechanical shaker, after which they were centrifuged and filtered through a Buchner funnel, using Whatman No. 1 filter paper. Ethanol was again removed through evaporation under reduced pressure at 40°C and the filtrate frozen at -80°C.

All fractions of the frozen, solid plant extract materials were crushed into smaller fragments, using a mortar and pestle. The crushed ice fragments were then transferred into conical glass flasks and the contents lyophilized using a Virtis benchtop freeze dryer (United Scientific, Gauteng, South Africa) for at least 72 hours. The dry materials were transferred into air-tight glass containers and stored in a desiccator until required for experimental purposes.

Chemical characterization of the herbal extracts

The herbal extracts were chromatographically profiled by means of ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), as well as liquid chromatography-ultraviolet (LC-UV) analytical methods. Solutions were prepared by adding 2 mL of methanol to 2 mg of freeze dried powder of the respective seed extracts. The resultant solutions were sonicated for 10 minutes and filtered through 0.2 µm syringe filters before UPLC-MS analysis. UPLC analyses were performed using a Waters ACQUITY Ultra Performance Liquid Chromatographic system with photodiode array detector (Waters, Milford, MA, USA). UPLC-MS separation was achieved with an ACQUITY UPLC BEH C₁₈ column (150 mm × 2.1 mm, 1.7 µm particle sizes, Waters), maintained at 40°C.

The chromatographic conditions are summarized in Table 1. Mass spectrometry was operated in negative ion electrospray mode. Nitrogen (N₂) was used as desolvation gas and the source temperature was set at 100°C.

Assessment of intestinal tissue integrity

Lucifer Yellow (LY) is a fluorescent stain (MW: 457.25 g/mol) employed as a para-cellular transport marker for assessing the integrity of the mounted jejunum tissue over a period of 2 hours. A 50 mL solution was prepared, containing 2.5 mg of LY in Krebs-Ringer bicarbonate (KRB) buffer. Transport studies were conducted on this LY solution in the apical-to-basolateral (AP-BL) direction. Fluorescence of the transported LY was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm by means of a fluorescence microplate reader (Spectramax Paradigm® multi-mode detection platform reader). The fluorescence data were processed, and the apparent permeability coefficient (P_{app}) of LY calculated to determine whether the intestinal tissue's integrity had been maintained for the duration of the transport experiment, in accordance with previously published P_{app} values (27,28).

Bi-directional transport studies

The gastrointestinal tract (GIT) of a pig was promptly removed from the carcass after slaughtering at the abattoir. A segment (± 30 cm) of the proximal jejunum was excised and thoroughly rinsed with ice-cold KRB buffer. The jejunum segment was then submerged in ice-cold KRB buffer, placed on ice in a cooler box, and immediately brought to the laboratory, where the piece of intestine was pulled over a wetted glass tube. The serosal layer was cautiously removed by making a superficial incision along

the mesenteric border and by pulling the layer off from the intestinal membrane.

The remaining tissue on the glass rod was inspected for Peyer's patches, which are areas of lymphoid tissue in the small intestine wall that can affect drug absorption, negatively impacting the transport results (29). These patches were excluded from the tissue that was used for the transport studies. Following this, the mesenteric border was used as a guide to dissect the segment of tissue to produce a flat sheet. The dissected tissue was rinsed with cold KRB buffer to remove it from the glass tube onto heavy-duty filter paper.

The membrane tissue was cut into smaller segments (±2 cm wide) and a piece of each mounted in between the two half cells of six diffusion chambers of the Sweetana-Grass (Ussing type) diffusion apparatus. The tissue segments were kept moist with cold KRB buffer throughout the procedure. After mounting, a tissue surface area of 1.78 cm² was available in each chamber for transport of the model compound. The six securely assembled diffusion chambers were placed in the diffusion apparatus, linked to a heating block (37°C) and a carbogen (95% O₂; 5% CO₂) supply (30).

The transport studies conducted on RH-123 in both directions (apical-to-basolateral (AP-BL) and basolateral-to-apical (BL-AP)) were performed in triplicate. Pre-heated (37°C) KRB buffer solution (7 mL) was added to both half cells of the six diffusion chambers and left to incubate for 15 minutes to allow for the sample tissues to adapt to their new environment. The KRB buffer was then removed from the chambers and the intestinal tissue incubated with an RH-123 solution with or without the herbal extracts. For the transport experiments conducted in the AP-BL direction, a 10 μM RH-123 solution was

Table 1. Chromatographic conditions used for the chemical characterization of the four herbal extracts

| Parameter | <i>Harpagophytum procumbens</i> | | <i>Hoodia gordonii</i> | | <i>Leonotis leonurus</i> | | <i>Vitis vinifera</i> | | | | | |
|------------------------------|---|---------|------------------------|------------|--------------------------|----|-----------------------|---------|----|------------|----|----|
| Mobile phase | Solvent A: 0.1% formic acid in water; Solvent B: acetonitrile | | | | | | | | | | | |
| | Time (min) | A | B | Time (min) | A | B | Time (min) | A | B | Time (min) | A | B |
| | 0 | 85 | 15 | 0 | 85 | 15 | 0 | 90 | 10 | 0 | 95 | 5 |
| | 4 | 65 | 35 | 4 | 50 | 50 | 2 | 60 | 40 | 8 | 25 | 75 |
| Gradient conditions | 6 | 50 | 50 | 6 | 25 | 75 | 12 | 30 | 70 | 10 | 22 | 78 |
| | 7 | 20 | 80 | 9 | 10 | 90 | 14 | 5 | 95 | 15 | 10 | 90 |
| | 8 | Holding | | 11 | Holding | | 14.5 | Holding | | 15.5 | 95 | 5 |
| | 8.5 | 85 | 15 | 11.5 | 85 | 15 | 15 | 90 | 10 | | | |
| Flow rate (mL/min) | 0.4 | | 0.3 | | 0.4 | | 0.3 | | | | | |
| Injection volume (μL) | 2 | | 2 | | 5 | | 4 | | | | | |
| Desolvation temperature (°C) | 400 | | 450 | | 350 | | 350 | | | | | |
| Desolvation flow rate (L/h) | 600 | | 550 | | 500 | | 500 | | | | | |
| Capillary voltage (V) | 2450 | | 3500 | | 2450 | | 2500 | | | | | |
| Cone voltage (V) | 46 | | 45 | | 46 | | 45 | | | | | |
| Mass-to-charge ratio (m/z) | 100 - 1500 | | 100 - 1500 | | 100 - 1200 | | 80 - 1200 | | | | | |

prepared and subsequently mixed in a 1:1 ratio with the respective herbal extract, rendering a final solution concentration of 5 μM of RH-123. For testing, 7 mL of this RH-123 and herbal extract mixture was added to the apical side (donor chamber) of the mounted tissue sample in the diffusion chamber.

The transport experiments conducted in the BL-AP direction required two separate solutions each of the RH-123 and of the respective herbal extract. A 50 mL solution of RH-123 was prepared in KRB buffer with a final concentration of 5 μM , from which 7 mL was added to each acceptor chamber (i.e., the basolateral side of the mounted tissue). For the donor chamber (i.e., the apical side of the tissue sample), three different concentrations of each herbal extract were used. For testing, 7 mL of each extract sample was added to each donor chamber. The herbal concentrations chosen for use in this study were based upon the total recommended daily allowance (RDA) of each of the herbal extracts, as is widely available in the literature (31). Furthermore, Hellum et al (31) recommended the use of three test concentrations by dissolving the herbal extract in three different fluid volumes (53.0 L, 5.3 L, and 0.53 L). These respective volumes represented the herbal extract concentration in the extra-cellular fluid (and plasma), and also the superlative concentrations that are expected to appear in the small intestine (to test across a concentration range that's inclusive of the lowest and highest values that may occur in the GIT). Application of the solutions in the manner described above was specifically designed to mimic normal physiological processes and conditions in the body. All herbal extracts were added to the apical side of the intestine, similar to where the constituents being tested would be present in the lumen of the GIT after oral administration.

The trans-epithelial electrical resistance (TEER) was measured at the beginning and end of each transport study using a Warner Instruments® EC-825A epithelial voltage clamp (Serial nr 211). Samples (180 μL) were withdrawn from the acceptor chambers at 20, 40, 60, 80, 100, and 120 minutes after application of the experimental solutions to the donor chambers. Samples were loaded into Costar® 96-well plates (The Scientific Group, Randburg, South Africa). Each withdrawn sample volume was replaced with 180 μL pre-heated KRB buffer (in the case of AP-BL transport), or with 180 μL of the experimental solution (in the case of BL-AP transport). A SpectraMax Paradigm® multi-mode detection platform reader was used to fluorometrically determine the RH-123 concentration in each experimental transport sample. A validated fluorescence spectroscopic analytical method was used. The performance characteristics that were measured during validation of the analytical method included linearity, the limit of detection, the limit of quantification, precision, specificity, and accuracy (details available upon request).

A negative and a positive control test were part of the design of the experiment. The negative control consisted of RH-123 alone (5 μM) and its transport was measured in both directions across the intestinal tissue membrane. The positive control consisted of verapamil (100 μM), a known P-gp inhibitor (32,33). The bi-directional transport of RH-123 was measured in the presence of verapamil to serve as the reference value, against which any potential efflux related effects of the herbal extracts could be compared. The purpose of the control experiments in this study was to determine whether any observed effects were indeed as a result of the extract's ability to cause a modulation of enzyme activity and/or of P-gp related efflux, or whether the outcomes had resulted from external factors, or any other arbitrary interference.

Data processing and statistical analysis

Percentage transport

The percentage of transport was calculated at each sampling interval after a correction was made for dilution. The correction factor was applied to firstly compensate for the lost amount of RH-123 during sampling, and secondly to compensate for the dilution of the remaining RH-123, due to the KRB buffer replacement.

$$\%Transport = \frac{\text{Mean fluorescence value at specific time}}{\text{Mean fluorescence value of donor solution}} \times 100$$

Apparent permeability coefficient

The apparent permeability coefficient (P_{app}) values for RH-123 in the presence and in the absence of the herbal extracts were calculated from the analytical transport curves.

$$P_{app} = \frac{dQ}{dt} \left(\frac{1}{A \cdot 60 \cdot C_0} \right)$$

P_{app} is the apparent permeability coefficient ($\text{cm} \cdot \text{s}^{-1}$), dQ/dt is the permeability rate (thus the amount of permeated RH-123 per minute), A is the diffusion area of the membrane (pig intestinal tissue), and C_0 is the initial concentration (μM) of RH-123 (34).

Efflux ratio

The efflux ratio (ER) value indicates the extent to which RH-123 was effluxed and calculated as follows (35):

$$ER = \frac{P_{app}(BA)}{P_{app}(AB)}$$

$P_{app}(BA)$ represents the apparent permeability coefficient value for RH-123 transport in the basolateral-to-apical direction and $P_{app}(AB)$ represents the RH-123 permeation in the apical-to-basolateral direction.

Statistical analysis

Statistical analysis was performed on all experimental data obtained from the bi-directional transport studies.

The Tukey's honest significant difference (HSD) post hoc test was performed to determine whether any statistically significant differences were evident from comparing the transport values of the control group (RH-123 alone) with those of the experimental groups. The HSD was also applied to determine whether any statistically significant differences were evident from a comparison among the experimental groups within each of the three extract concentration groups. Differences between the control and experimental groups were considered to be statistically significant if $P \leq 0.05$. Furthermore, an analysis of variance (ANOVA) test, together with a "2-way table of descriptive statistics" and the "Brown-Forsythe test of homogeneity of variances" were performed to identify any statistical differences among the data being generated from the transport experiments.

Results

Chemical profiling of the herbal extracts

Results from the UPLC-MS chemical analyses of the four herbal extracts were indicative of the presence of certain

phytochemical constituents that are known chemical markers of the selected plant species (Figures 1-4) (36-38). The UPLC-MS and LC-UV chromatograms obtained for the *H. procumbens* extract (Figure 1) indicated the presence of harpagoside (MW = 493.146 g/mol), which was present in a concentration of 17.5 µg/mg dry weight of the extract. An oxypregnane steroidal glycoside (P57AS3 or P57, MW = 295.204 g/mol) was identified in the *H. gordonii* extract (Figure 2) and quantified as having a concentration of 6.2 µg/mg of the dry weight of the extract. The UPLC-MS chromatogram obtained for the *L. leonurus* extract (Figure 3) indicated the presence of narrulibanoside in a concentration of 35.4 µg/mg per dry weight of the extract. The presence of procyanidin B1 and procyanidin B2 (MW = 564.3246 g/mol) was confirmed in the *V. vinifera* seed extract (Figure 4).

Assessment of intestinal tissue integrity

Irvine et al (39) determined that in order to verify the integrity of a biological membrane, the P_{app} value of LY permeation should be in the range of 1×10^{-7} cm/s to

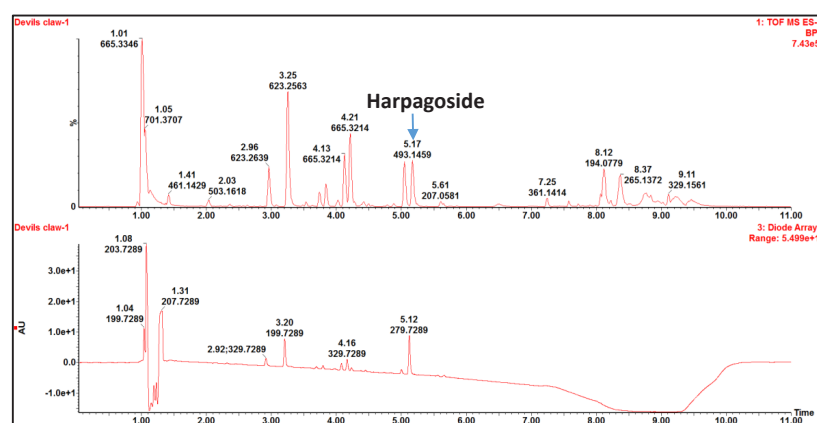


Figure 1. UPLC-MS and LC-UV chromatograms of *Harpagophytum procumbens* extract, indicating the presence of the phytochemical marker molecule, harpagoside.

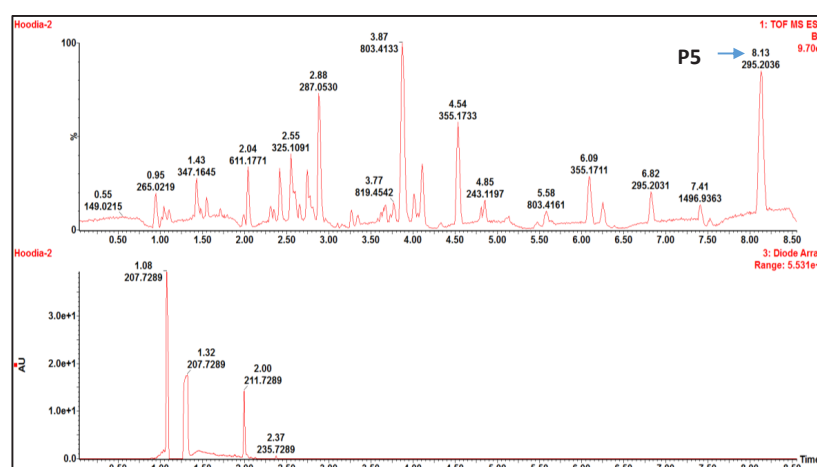


Figure 2. UPLC-MS and LC-UV chromatograms of *Hoodia gordonii* extract, indicating the presence of the oxypregnane steroidal glycoside active constituent (P57).

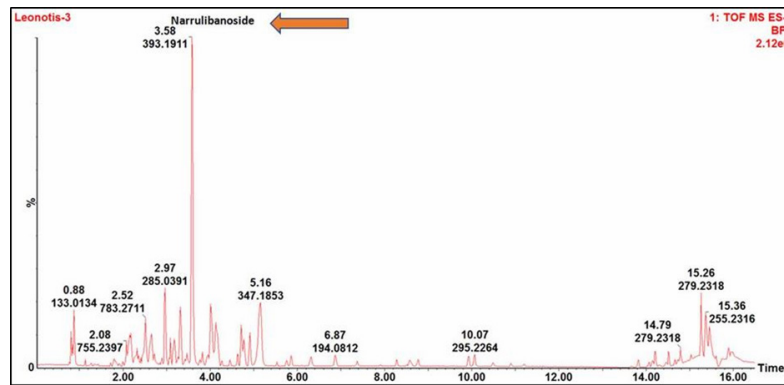


Figure 3. UPLC-MS chromatogram of *Leonotis leonurus* extract, indicating the presence of narrulibanoside.

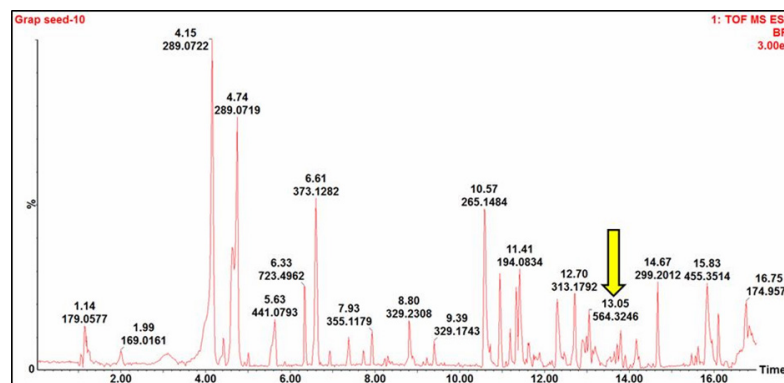


Figure 4. UPLC-MS chromatogram of *Vitis vinifera* seed extract, indicating the presence of procyanidin B1 and procyanidin B2.

7×10^{-7} cm/s. The average P_{app} value obtained for LY permeation in this study was 3.097×10^{-7} cm/s, indicating tissue integrity had been maintained throughout the duration of the transport experiments.

Bi-directional transport studies

Figure 5 illustrates the average P_{app} values obtained for the bi-directional transport of RH-123 in the presence of the *H. procumbens* extract at the three different concentrations.

The calculated average P_{app} values, as illustrated in Figure 5, indicate that a concentration-dependent decrease in RH-123 transport in the AP-BL (absorptive) direction had occurred in the presence of the *H. procumbens* extract, when compared to the negative control. Conversely, there was a slight concentration-dependent increase in RH-123 transport in the BL-AP (secretory) direction, when compared to the control group. However, these observed changes in permeation of RH-123 in the presence of the *H. procumbens* extract were statistically insignificant.

Figure 6 illustrates the average P_{app} values for the bi-directional transport of RH-123 in the presence of the *H. gordonii* extract at three different concentrations.

Figure 6 shows that RH-123 transport across the excised intestinal tissues had increased in a concentration dependent manner in the absorptive (AP-BL) direction.

However, only the highest concentration of the *H. gordonii* extract had statistically significantly increased the permeation of RH-123 in the absorptive direction. Conversely, a concentration dependent decrease in the secretory (BL-AP) direction of RH-123 was observed in the presence of the *H. gordonii* extract. Again, only the highest concentration of the *H. gordonii* extract showed a statistically significant decrease in RH-123 efflux, similar to that of the positive control (RH-123 and verapamil combination).

Figure 7 illustrates the average P_{app} values for the bi-directional transport of RH-123 in the presence of the *L. leonurus* extract at three different concentrations.

Figure 7 shows a decrease in RH-123 transport being mediated by the *L. leonurus* extract in the AP-BL direction, when compared to the negative control (RH-123 alone), in an inverse correlation with the applied concentration. In the BL-AP direction, only the highest concentration of the *L. leonurus* extract showed an increase in the secretory transport of RH-123, when compared to the negative control. The medium and low concentrations of the *L. leonurus* extract did not have any significant effect on RH-123 transport in the BL-AP direction, when compared to the negative control.

Figure 8 illustrates the average P_{app} values for the bi-

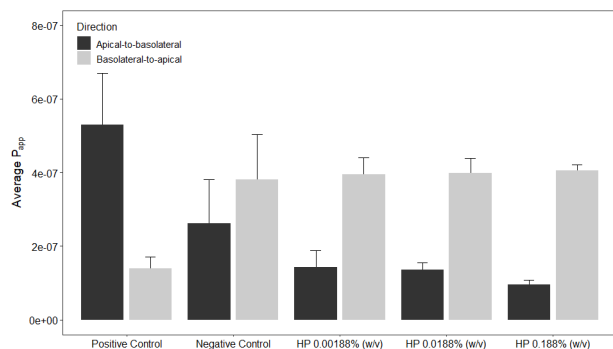


Figure 5. Apparent permeability of Rhodamine 123 across excised pig jejunum tissue in the presence of *Harpagophytum procumbens* (HP) extract at three concentrations. Data are expressed as the mean apparent permeability coefficient (P_{app}) values \pm standard deviation; $n = 3$.

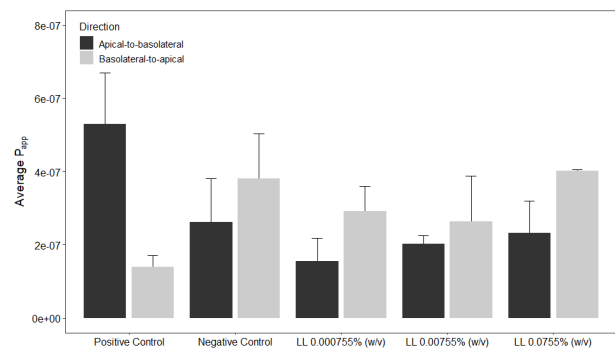


Figure 7. Apparent permeability of Rhodamine 123 across excised pig jejunum tissue in the presence of *Leonotis leonurus* (LL) extract at three concentrations. Data are expressed as the mean apparent permeability coefficient (P_{app}) values \pm standard deviation; $n = 3$.

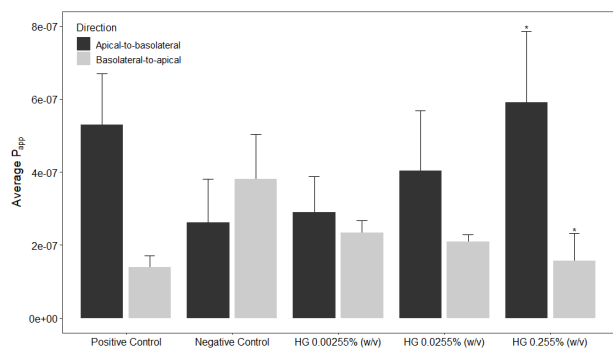


Figure 6. Apparent permeability of Rhodamine 123 across excised pig jejunum tissue in the presence of *Hoodia gordonii* (HG) extract at three concentrations. Data expressed as the mean apparent permeability coefficient (P_{app}) values \pm standard deviation; * $P < 0.05$, compared to the positive control group, $n = 3$.

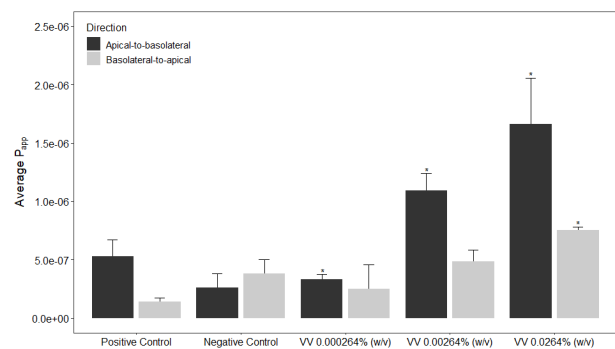


Figure 8. Apparent permeability of Rhodamine 123 across excised pig jejunum tissue in the presence of *Vitis vinifera* (VV) extract at three concentrations. Data expressed as the mean apparent permeability coefficient (P_{app}) values \pm standard deviation; * $P < 0.05$, compared to the positive control group, $n = 3$.

directional transport of RH-123 in the presence of the *V. vinifera* seed extract at three different concentrations.

Figure 8 shows that the *V. vinifera* seed extract had mediated a concentration dependent increase in both the absorptive (AP-BL) and secretory (BL-AP) transport of RH-123, when compared to the negative control (RH-123 alone). Statistically significant differences in the extent of RH-123 transport, compared to the control, had occurred at all three *V. vinifera* seed extract concentrations in the AP-BL direction, as well as at the highest extract concentration in the BL-AP direction.

Evaluation of efflux ratio values

It is evident from the ER results, as depicted in Table 2 that *H. procumbens* had resulted in a concentration dependent induction of RH-123 efflux, with ER values well above 1. Higher concentrations of the *H. procumbens* extract resulted in an increased RH-123 efflux, as indicated by the higher ER values. Conversely, the *H. gordonii* and *V. vinifera* extracts showed a slight concentration dependent inhibition of RH-123 efflux, with ER values below 1. Higher concentrations of the *H. gordonii* and *V. vinifera*

extracts led to increased absorptive uptake of RH-123. The *L. leonurus* extract showed an induction of RH-123 efflux at low and high concentrations, whereas the medium concentration showed a negligible effect on the modulation of RH-123 efflux.

Comparison and evaluation of TEER

The TEER values did not differ notably for most of the herbal extracts investigated during this study. A significant change in TEER (38.10% decrease) was, however, observed in the presence of *H. gordonii* at the high concentration (0.255% w/v) in the AP-BL direction. Moderately lower TEER values (15.62% and 19.43%) were observed in the presence of the medium (0.00755% w/v) and high (0.0755% w/v) *L. leonurus* concentrations, respectively, in the BL-AP direction. An overall reduction in TEER (between 24.92% and 36.56%) was observed in the presence of the *V. vinifera* seed extract at all three concentrations (0.000264, 0.00264 and 0.0264% w/v) in the AP-BL direction, suggesting that tight junctions had been modulated.

Table 2. Efflux ratio values calculated for RH-123 in the presence of each of the four herbal extracts at the three chosen concentrations

| Herbal extract | Low concentration | Medium concentration | High concentration |
|---------------------------------|-------------------|----------------------|--------------------|
| <i>Harpagophytum procumbens</i> | 2.75 | 2.93 | 4.2 |
| <i>Hoodia gordonii</i> | 0.8 | 0.52 | 0.27 |
| <i>Leonotis leonurus</i> | 1.96 | 1.3 | 1.73 |
| <i>Vitis vinifera</i> | 0.96 | 0.45 | 0.45 |

Discussion

The aim of this study was to investigate and identify potential pharmacokinetic interactions that may occur between four selected herbal extracts and a model compound, Rhodamine 123, a known P-gp substrate when administered concomitantly.

It was evident from the bi-directional transport results that changes in the directional transport of RH-123 had occurred in the presence of the herbal extracts. When efflux occurs (i.e., secretory transport increases due to P-gp induction), a lesser amount of the drug is expected to traverse the epithelium through absorption, due to the active pumping of the molecules from within the cells to the initial apical side (40). This increase in efflux would thus reduce the bioavailability of any drug, when administered concomitantly with an efflux inducing compound. This phenomenon was observed for the *H. procumbens* extract at low, medium, and high concentrations, as well as for *L. leonurus* at high concentrations. Since TEER values showed only negligible fluctuations in both directions of transport (AP-BL and BL-AP), it was concluded that the *H. procumbens* extracts had little to no effect on tight junction modulation. The slight decrease in TEER values in the presence of medium and high *L. leonurus* extract concentrations in the BL-AP direction were in accordance with the slight increase in secretory RH-123 transport that was observed with *L. leonurus* at a high concentration. The calculated ER values (>1) in the presence of the respective *L. leonurus* extract concentrations (Table 2) suggest that the *L. leonurus* extract had mediated an induction of P-gp related efflux upon reaching a specific baseline concentration, as was evident for secretory (BL-AP) RH-123 transport in the presence of the highest *L. leonurus* extract concentration (Figure 7). Additional studies regarding the different transport mechanisms involved would be required to determine at exactly which critical *L. leonurus* concentration the turning point in transport mechanisms occur.

On the other hand, a reduction in P-gp related efflux, as a result of P-gp inhibition, may lead to an increase in the bioavailability of co-administered compounds, as was evident from the transport results of RH-123 in the presence of the *H. gordonii* extracts. The significant decrease in TEER (38.10%) of the membrane tissue in the AP-BL direction, when the highest concentration of the *H. gordonii* extract was applied, suggested that para-cellular uptake *via* opened tight junctions may have contributed

towards the increased uptake of RH-123. Bock et al (41) previously reported that an ER below 1 was indicative of absorptive uptake, resulting from a reduced efflux. The calculated ER values for the three different *H. gordonii* extract concentrations (Table 2) thus suggest that the moderately higher RH-123 transport values had resulted from P-gp related efflux inhibition, rather than from tight junction modulation. Regarding the P_{app} values, the transport of RH-123 in the presence of *H. gordonii* has been significantly higher in the absorptive than in the secretory direction (Figure 6). These results corresponded with the findings of Vermaak et al (42) and Madgula et al (43), where the *H. gordonii* extracts were able to inhibit P-gp related efflux. The low and medium concentrations of the *L. leonurus* extract possibly inhibited efflux, based upon the calculated P_{app} values for BL-AP transport, as they were lower than that of the negative control (Figure 7). In the case of *V. vinifera*, the increased absorptive and secretory transport results of RH-123 indicated that more than one transport mechanism might have been at play, namely an increased absorptive uptake, either due to efflux inhibition or as a result of tight junction opening. Observed ER values (Table 2) indicated that the transport has been subjected to increased absorptive uptake due to RH-123 efflux inhibition (41). In a study performed by Zhao et al (44), it was found that the *V. vinifera* seed extract had the ability to inhibit P-gp activity, with a resultant increase in the intracellular accumulation of RH-123. The findings from this bi-directional transport study were in accordance with those of Zhao et al (44). However, the *V. vinifera* seed extract may have had effects on transport mechanisms, other than P-gp related efflux. An overall decrease in the TEER values of the excised jejunum tissues during the transport study in the AP-BL direction, indicated that a pronounced opening of tight junctions had been mediated by the *V. vinifera* seed extract. A study conducted by Takizawa et al (45) illustrated that RH-123 could also be transported by means of para-cellular diffusion across membranes. It could, therefore, be possible that the decrease in TEER values in this study may have also mediated an increase in the diffusion of RH-123 across the membrane. However, additional studies would be required to elucidate the primary transport mechanism of RH-123.

Conclusion

The current study demonstrated various membrane

permeation modulating effects by four selected herbal extracts, i.e., *Harpagophytum procumbens*, *Hoodia gordonii*, *Leonotis leonurus*, and *Vitis vinifera* on a model compound, Rhodamine 123. A study on CYP450 enzymes may indicate whether co-administration of any of the herbal extracts would influence the metabolic enzyme activity and thus the bioavailability of substrate drugs. Additional studies could be performed with extracts derived from different parts of the plants, e.g. the leaves, bark, or flowers. Such studies could also investigate the effects of using different methods of herbal extract preparation, e.g. infusion in different solvents, decoctions, and teas. It is highly recommended that *in vivo* bioavailability studies should be conducted in conjunction with *ex vivo* studies to verify the clinical significance of the results obtained.

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Authors' contributions

JHH and JDS conceptualized, funded and supervised the project. AV assisted with sourcing plant materials, supervised the chromatographic analysis and edited the manuscript. CJ performed the experiments and analyzed the data. BP wrote the paper. DJS and JHH revised the paper. All authors read and confirmed publication of the paper.

Conflict of interests

The authors declare no conflict of interests.

Ethical considerations

The use of excised pig jejunum intestinal tissue obtained from pigs slaughtered at an abattoir was approved by the Ethics Committee (AnimCare) of the North-West University (NWU00025-15-A5).

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