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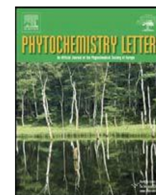

Effect of simulated gastrointestinal conditions and epithelial transport on extracts of green tea and sage

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ABSTRACT

Few in vitro screening studies on the biological activities of plant extracts that are intended for oral administration consider the effect of the gastrointestinal system. This study investigated this aspect on extracts of Camellia sinensis (green tea) and Salvia officinalis (sage) using antimicrobial activity as a model for demonstration. Both the crude extracts and their products after exposure to simulated gastric fluid (SGF) as well as simulated intestinal fluid (SIF) were screened for antimicrobial activity. The chromatographic profiles of the crude plant extracts and their SGF as well as SIF products were recorded and compared qualitatively by means of high performance liquid chromatography coupled to mass spectrometry. The effect of epithelial transport on the crude plant extracts was determined by applying them to an in vitro intestinal epithelial model (Caco-2). The crude extracts for both plants exhibited reduced antimicrobial activity after exposure to SGF, while no antimicrobial activity was detected after exposure to SIF. These results suggested chemical modification or degradation of the antimicrobial compounds when exposed to gastrointestinal conditions. This was confirmed by a reduction of the peak areas on the LC–UV–MS chromatograms. From the chromatographic profiles obtained during the transport study, it is evident that some compounds in the crude plant extracts were either not transported across the cell monolayer or they were metabolised during passage through the cells. It can be deduced that the gastrointestinal environment and epithelial transport process can dramatically affect the chromatographic profiles and biological activity of orally ingested natural products.
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1. Introduction

The popularity of tea is evident from the fact that it is the most widely consumed beverage next to water worldwide. Camellia sinensis (L.) Kuntze, a member of the Theaceae family, is indigenous to southern and eastern Asia and commercial cultivation has spread to India, Sri Lanka, Malaysia, Indonesia and Africa. Numerous studies have shown that green tea possesses several biological properties including antioxidant, anti-inflammatory, antiviral, antidiabetic and anticarcinogenic effects amongst others (Van Wyk and Wink, 2004; Sharangi, 2009). Although the health benefits of green tea has been ascribed to its strong antioxidant potential, some studies have also reported on the antimicrobial activity of green tea and its constituents. It has been documented that tea catechins such as (-)-epigallocatechin-3-gallate (EGCG) are responsible for both the antioxidant and antimicrobial effects of green tea. EGCG exhibits the highest antioxidant as well as antimicrobial effect with smaller contributions from the other catechins, probably due to the fact that only small amounts are present (Hamilton-Miller, 1995; Cabrera et al., 2006).

Salvia officinalis L. (Lamiaceae) is commonly known as sage and is used frequently as a culinary herb. S. officinalis is native to the Mediterranean rim and is cultivated in many other countries. S. officinalis is a popular remedy to treat gingivitis and mucosal inflammation of the mouth and throat and is also used to treat gastrointestinal disturbances such as flatulence and diarrhoea (Blumenthal et al., 2000; Van Wyk and Wink, 2004). The essential oil is considered among the most important of the antimicrobial agents from sage and Delamare et al. (2007) reported remarkable bacteriostatic and bactericidal activities against several micro-organisms. In addition, oleanolic acid isolated from an extract of sage also exhibited some antimicrobial activity (Horiuchi et al., 2007).

Due to the need for the development of novel drugs there has been a renewed interest in ethnopharmacological research worldwide. Studies are generally of a screening nature and investigate biological activities such as antioxidant, antibacterial, anti-inflammatory and antifungal properties, usually based on ethnobotanical leads. The effect that the gastrointestinal environment may have on these orally consumed extracts is often not

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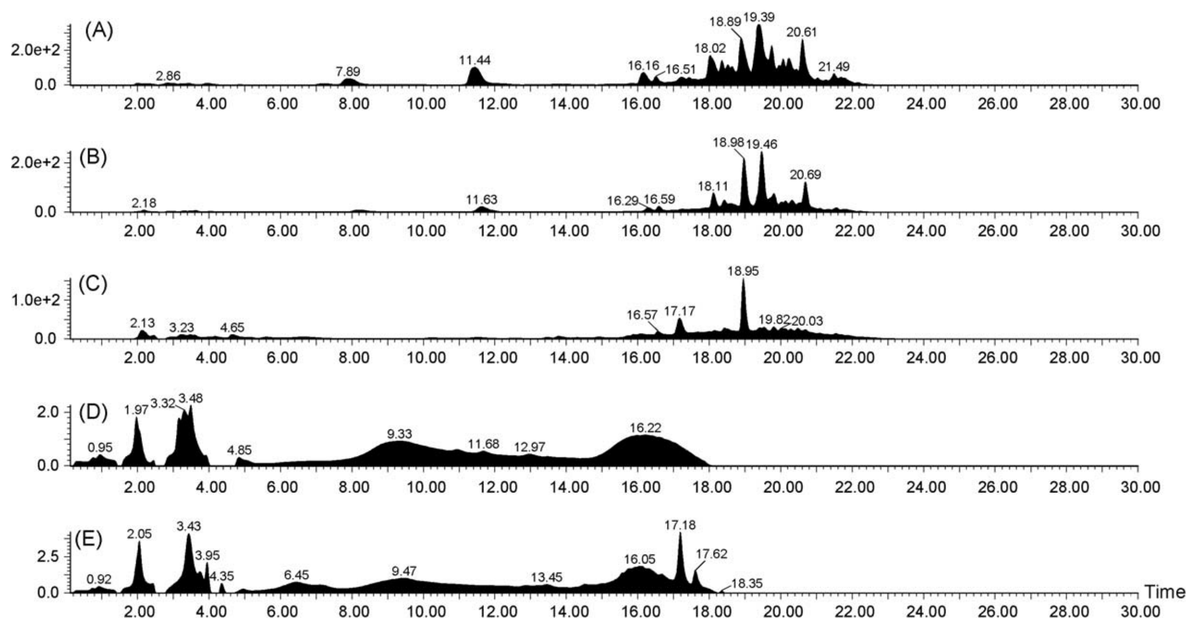

Fig. 1. LC–UV chromatograms of C. sinensis crude aqueous extract, simulated gastric fluid and simulated intestinal fluid products, as well as simulated gastric fluid and simulated intestinal fluid control samples. (A) Crude extract, (B) simulated gastric fluid product, (C) simulated intestinal fluid product, (D) simulated gastric fluid control, and (E) simulated intestinal fluid control.

explored even though it is well known and accepted in biopharmaceutics that the gastrointestinal system plays a considerable part in the ultimate bioavailability of medicines (Ashford, 2007). The human gastrointestinal tract transforms orally consumed substances into absorbable molecules and waste products by means of a digestive process. The fluids present in the gastrointestinal system contain various substances and enzymes which aid in the digestion process. The stomach has a pH between 1 and 2 due to the secretion of hydrochloric acid and contains the digestive enzyme pepsin. The pH of the small intestine ranges between 5.1 and 7.5 and contains numerous digestive enzymes in the lumen and in the epithelial cells lining the gastrointestinal tract. Unfortunately, these natural digestive processes and barrier mechanisms in the gastrointestinal tract also negatively affect oral drug delivery (Mader, 1996; Hamman et al., 2005). In this study, the effect of simulated gastrointestinal conditions and the intestinal epithelial cell transport process on extracts from two commercially important medicinal plants were investigated.

2. Results and discussion

The antimicrobial activity (minimum inhibitory concentration, MIC) results for C. sinensis are summarised in Eloff (1998); Table 1 (see supplementary data). Sterile water and broth with bacterial culture exhibited growth indicating viability of the microorganisms. The positive control, ciprofloxacin, was active against all the test organisms. The wells of the Tryptone Soya Broth (TSB) control column remained clear after incubation indicating sterility of the TSB.

Lee et al. (2003) reported antimicrobial activity of 3.1 mg/ml against Staphylococcus aureus, 50 mg/ml against Enterococcus faecalis and 12.5 mg/ml against Escherichia coli ATCC strains for a 10% tea broth. In this study, antimicrobial activity of 1–4 mg/ml was observed for the crude extracts of C. sinensis. After exposure to SGF, the activity of both the water and methanol extracts decreased to 16 mg/ml against S. aureus and to 8 mg/ml against E. coli, while they were completely ineffective against Enterococcus faecalis and Proteus vulgaris. These results indicated that chemical modification of the antimicrobially active compounds in the extracts occurred only to some extent during exposure to SGF because some antimicrobial activity was still evident. On the other hand, no antimicrobial activity was evident for the SIF products suggesting that the antimicrobially active compounds in the extracts were completely modified or degraded during exposure to the SIF. These in vitro results indicated that the antimicrobial compounds contained in green tea are possibly inactivated by gastrointestinal conditions, which will most probably affect its systemic antibiotic action.

The chromatograms of C. sinensis crude aqueous extract, SGF as well as SIF products and control samples are shown in Fig. 1. The compounds detected at retention times of 7.89, 16.16 and 21.49 min were only present in the crude extracts, while they were not detected in the SGF or SIF products. This indicated possible degradation or chemical modification of these compounds after exposure to simulated gastrointestinal conditions. The peak areas for compounds detected at retention times of 11.44–11.63, 18.02–18.11, 19.39–19.46 and 20.61–20.69 min in the crude extracts decreased after exposure to SGF and none of these compounds were detected in the SIF products. These results from the chromatographic profiles are in agreement with the results obtained from the antimicrobial activity tests. This indicated that compounds with antimicrobial activity will still be available from C. sinensis extracts to some extent for absorption from the stomach. However, it is most likely that complete degradation will occur in the small intestines with potentially no systemic antimicrobial effect from the part of the C. sinensis extracts that reach the small intestines, which is usually the main gastrointestinal segment for absorption of xenobiotics.

Fig. 2 shows the chromatograms for the samples obtained from the in vitro epithelial transport study (Caco-2) representing the crude aqueous extract of C. sinensis in the apical chamber (A) and the basolateral chamber (B). The comparison of selected peak areas and the calculated percentages of these compounds transported across the Caco-2 cell monolayers for the crude aqueous extract and SGF as well as SIF fluid products are displayed in Table 2 (see supplementary data). In general, the epithelial transport results obtained for C. sinensis indicated fairly good transport for the compounds that survived the exposure to simulated gastrointestinal conditions with few compounds that were not transported or degraded during their passage through the cell monolayer. For

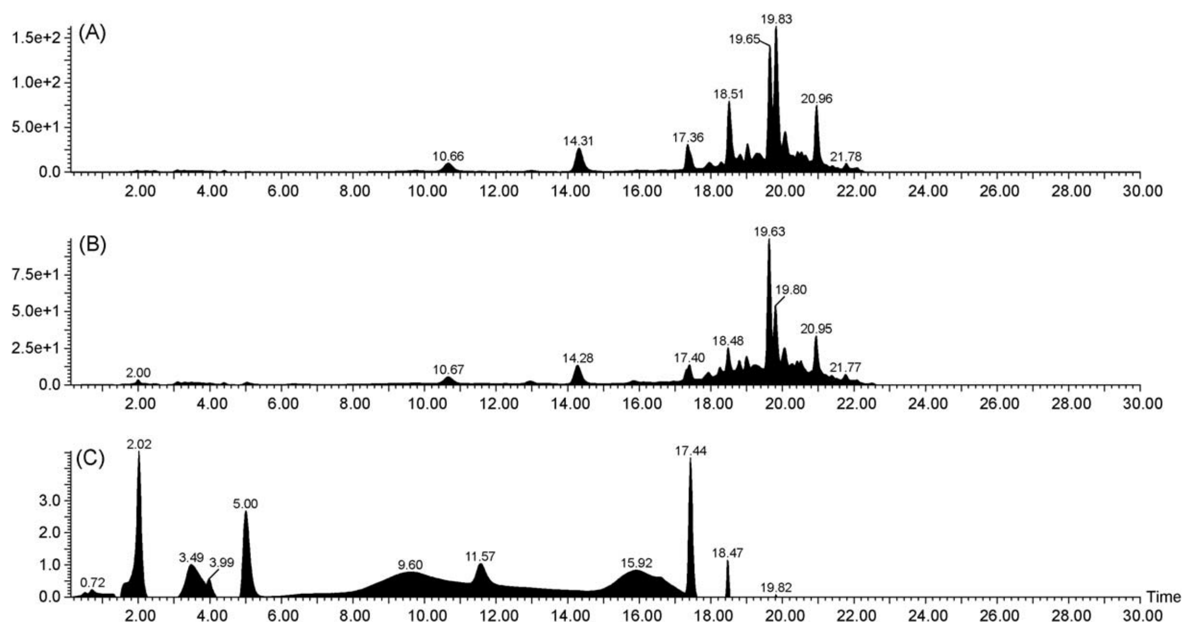

Fig. 2. LC–UV chromatograms of C. sinensis crude aqueous extract. (A) Apical chamber, (B) basolateral chamber after 4 h, and (C) transport medium (DMEM) control.

example, 34.80% of the compound at retention time 19.82–19.83 min from the crude aqueous extract was transported across the cell monolayers while 79.78% of the compound at retention time 19.63–19.65 min was transported across the cell monolayers as calculated from the peak areas on the chromatograms. Furthermore, the compound at retention time 19.81 min was not detected in the sample withdrawn from the basolateral side indicating that this compound was either not transported or possibly metabolised to levels beyond the detection limit of the apparatus. Kunhle et al. (2000) reported that catechin and epicathecin, flavanoids found in green tea, are extensively metabolised and conjugated by glucuronidation and O-methylation during transfer across the enterocytes. The results therefore correlate with a study by Feng (2006) suggesting that intestinal metabolism and chemical degradation amongst others is responsible for the low bioavailability of certain tea catechins in animals and most likely also in humans.

The antimicrobial activity (MIC) results for S. officinalis are summarised in Table 3 (see supplementary data). Weckesser et al. (2007) previously reported antimicrobial activity of 100 mg/ml for a supercritical carbon dioxide extract of S. officinalis dissolved in ethanol against E. faecalis and three strains of S. aureus, while no activity was reported for E. coli at the concentrations tested (100–0.2 mg/ml). In this study, the crude S. officinalis extracts exhibited some antimicrobial activity (2–8 mg/ml) against the four pathogens tested. After exposure to SGF, the antimicrobial activity of the extracts was decreased to �16 mg/ml, indicating chemical modification or degradation of the antimicrobially active

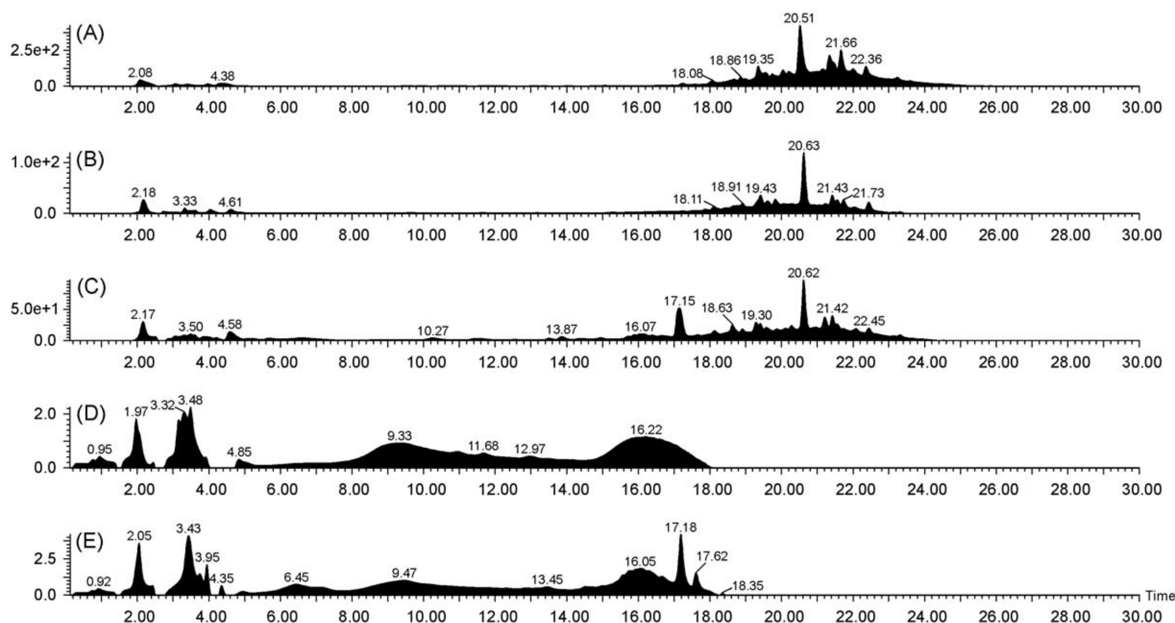

Fig. 3. LC–UV chromatograms of S. officinalis crude aqueous extract, simulated gastric fluid and simulated intestinal fluid products, as well as simulated gastric fluid and simulated intestinal fluid control samples. (A) Crude extract, (B) simulated gastric fluid product, (C) simulated intestinal fluid product, (D) simulated gastric fluid control, and (E) simulated intestinal fluid control.

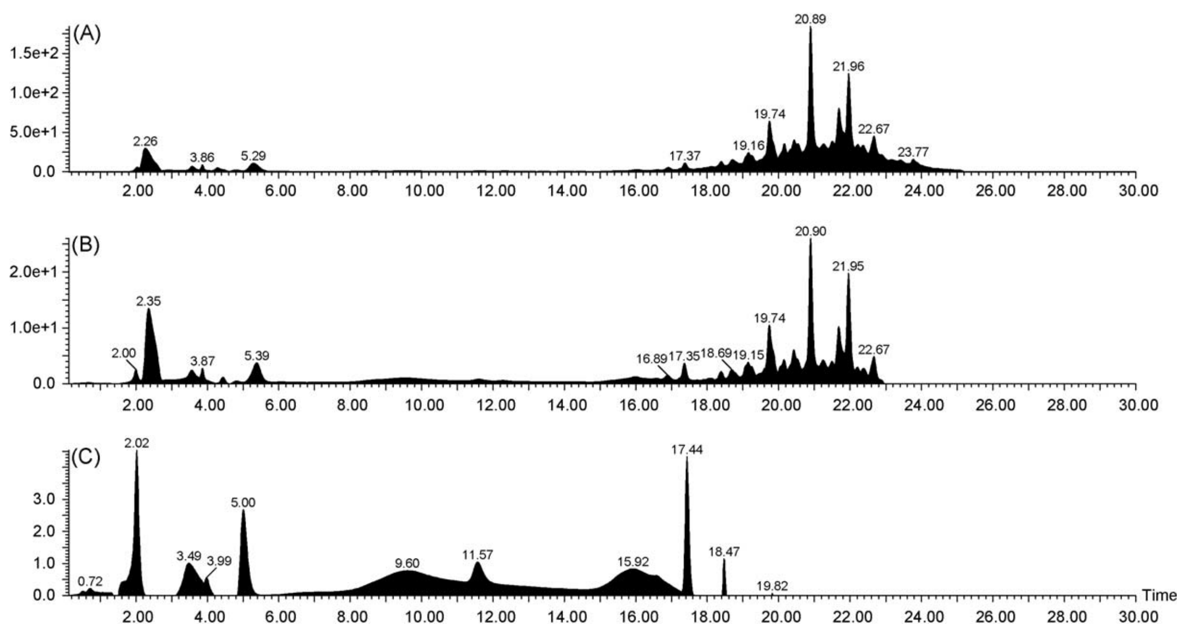

Fig. 4. LC–UV chromatograms of S. officinalis crude aqueous extract. (A) Apical chamber, (B) basolateral chamber after 4 h, and (C) transport medium (DMEM) control.

compounds in the extracts to a relatively high extent. No antimicrobial activity was observed after exposure to SIF. These results indicated that the active antimicrobial constituents of S. officinalis will probably be deactivated in the gastrointestinal tract after oral ingestion and thus it is unlikely that it will exhibit a systemic antibiotic action.

The chromatograms of S. officinalis crude aqueous extract, SGF as well as SIF products and control samples are shown in Fig. 3. The peak areas of the compounds detected at retention times of 20.51–20.63 and 22.36–22.45 min decreased after exposure to SGF and decreased further after exposure to SIF. However, the peak area of the compound at retention time 19.30–19.43 min decreased more after exposure to SGF than after exposure to SIF indicating greater sensitivity and susceptibility to degradation in the conditions of the stomach as compared to those of the small intestines. These results from the chemical composition of the S. officinalis extracts are in agreement with the antimicrobial results and confirm degradation of compounds that are most probably responsible for the antimicrobial activity after exposure to simulated gastrointestinal conditions.

Fig. 4 depicts the chromatograms for the samples obtained from the in vitro epithelial transport study representing the crude aqueous extract of S. officinalis. Although the chromatograms are qualitatively similar, the peak area of selected compounds in the extract decreased significantly as shown in Table 4 (see supplementary data). The percentage transport for selected compounds from S. officinalis ranged from 15.10 to 19.47% for the crude extract, while it was 34.08 to 40.06% for the SGF product and only 4.48 to 11.11% for the SIF product. These results indicated that some of the compounds in the extracts were either metabolised within the enterocytes or not transported across the Caco-2 cell monolayer. In general, this in vitro transport study indicated a relatively low absorption extent of the compounds in S. officinalis extracts with potential low bioavailabilities, which may impact on its systemic effects.

In conclusion it is evident from this study that extracts from the two selected plants are dramatically affected by exposure to simulated gastrointestinal conditions both in terms of biological activity (i.e. antimicrobial activity) and their chromatographic profiles. It is further evident that the transport process across intestinal epithelial cells affects certain compounds in the extracts that may even further impact on the systemic effects of these plants. This proves that results from screening assays of medicinal plants may lead to overestimation of antimicrobial activity if the effect of the gastrointestinal system is not taken into consideration. However, it should also be mentioned that weak antimicrobial activity noted during an in vitro screening assay may lead to underestimation, since compounds are sometimes activated when exposed to gastrointestinal tract conditions (Vermaak et al., in press).

3. Experimental

3.1. Plant materials

C. sinensis (green tea) teabags were purchased at a retail shop in Pretoria (South Africa). S. officinalis (sage) plants from the Margaret Roberts range were purchased at a nursery in Pretoria (South Africa) and the leaves were air-dried. Retention specimens were deposited in the Department of Pharmaceutical Sciences, Tshwane University of Technology.

3.2. Extract and final product preparation

The dried plant material was ground into a fine powder and extracted with H_2O and MeOH in a water bath (Scientific 32 l) at 40 8C for 3 h. This was repeated 3x to maximise extraction efficiency. The MeOH extracts were dried by evaporation and the H_2O extracts were freeze-dried. These extracts were subjected to simulated gastrointestinal conditions using a six-station Pharmatest Type PTW5 dissolution apparatus set up according to the British Pharmacopoeia specifications (BP, 1988). The paddle apparatus was set to 37 8C with a paddle speed of 150 rpm. The simulated fluids were prepared as prescribed in the United States Pharmacopoeia (USP, 1990). The composition of SGF was slightly modified for the purposes of this study, namely HCl was replaced with TFA (Sigma–Aldrich) as it is volatile thereby eliminating the possible interference of HCl with subsequent antimicrobial experiments. The dissolution time of 2 h for exposure to SGF and 6 h for exposure to SIF were selected to simulate the transit time of ingested substances in the individual gastrointestinal segments. After 900 ml of dissolution medium were added to the

dissolution flasks and the temperature was stabilised at 37 8C, the paddles were lowered and 500 mg of each plant extract were added to the different gastrointestinal fluids. After the appropriate time for each simulated fluid had elapsed, the total contents of the flasks were dried by evaporation. The extracts were re-dissolved in 30 ml of distilled H_2O, 10 ml of MeOH were added and the mixture was sonicated (Sonorex digital 10p) for 10 min at 37 8C and 100% power to dissolve the solids. Proteins (added as enzymes in the simulated fluids) were removed by complexation with the addition of 30 ml of CH_3CN placed in flasks in a water bath (Scientific 32 l) at 60 8C for 10 min. The products were filtered (Whatman[1] no. 41) and dried by evaporation. For each plant, crude H_2O and MeOH extracts, H_2O and MeOH SGF products as well as H_2O and MeOH SIF products were therefore obtained for testing purposes.

3.3. Chromatographic profiling

The detection of chemical constituents was performed using a Waters Alliance 2690 HPLC system (Phenomenex Aqua C18, 4 u, 250 mm × 2 mm) coupled to a 996 PDA detector and a Waters API Qautro MS detector. The MS detector was operated in electron impact mode with the capillary voltage at 3.5 kV and the cone voltage at 15 (ES^+) and 25 (ES^-). The flow rate of the HPLC was 0.3 ml/min, the nebuliser flow rate 50 l/h and the desolvation gas flow rate 380 l/h. The mobile phase used consisted of 0.1% HC_2OH and CH_3CN and gradient elution was used over a 40 min period (Table 5, see supplementary data). The test samples were dissolved in distilled water to a concentration of 50 mg/ml. The Masslynx™ (Version 4.0) software package and Microsoft[1] Excel (2003) were used for data analysis. For the sake of brevity the LC–MS results which confirmed the LC–UV results are omitted from this paper.

3.4. Transport studies (Caco-2 cell monolayers)

Caco-2 cells were grown in 25 cm^3 cell culture flasks (Corning Costar Corporation, USA) in culture medium consisting of Dulbecco's Modified Eagles Medium (DMEM) (Gibco, Auckland, NZ), supplemented with 1% pen/strep fungizone mixture (Highveld Biological, Lyndhurst, RSA), 10% foetal bovine serum (Delta Bioproducts, Kempton Park, South Africa), and 1% non-essential amino acids (Gibco, Auckland, NZ). The cells were seeded on tissue culture-treated polycarbonate filters in Costar Transwell six-well plates (Corning Costar Corporation, USA) at a density of 1.0×10^4 cells/ml. The plates were kept in an incubator at a temperature of 37 8C in an atmosphere of 95% air and 5% CO_2. The experiments were performed between 19 and 23 days after seeding on cell monolayers that had reached transepithelial electrical resistance (TEER) values above 200 Vm/cm^2 (Brown et al., 2003; Hamman et al., 2003). The extracts and products were dissolved in DMEM to a concentration of 200 mg/2.5 ml. Pre-warmed (37 8C) sample solution (2.5 ml) was introduced into the apical chamber and 2.5 ml of buffered DMEM into the basolateral chamber. For comparative analysis, some of the initial sample was retained. The plates were incubated at 37 8C for 4 h whereafter samples were drawn from the basolateral chamber. The samples were analysed using LC–UV–MS under the same conditions as described previously.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2009.05.004.

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