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# Permeability of Rosmarinic acid in *Prunella vulgaris* and Ursolic acid in *Salvia officinalis* Extracts across Caco-2 Cell Monolayers

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## Abstract

**Ethnopharmacological relevance—**Rosmarinic acid (RA), a caffeic acid-related compound found in high concentrations in *Prunella vulgaris* (self-heal), and ursolic acid (UA), a pentacyclic triterpene acid concentrated in *Salvia officinalis* (sage), have been traditionally used to treat inflammation in the mouth, and may also be beneficial for gastrointestinal health in general.

**Aim of the study—**To investigate the permeabilities of RA and UA as pure compounds and in *P. vulgaris* and *S. officinalis* ethanol extracts across human intestinal epithelial Caco-2 cell monolayers.

**Materials and methods—**The permeabilities and Phase II biotransformation of RA and UA as pure compounds and in herbal extracts were compared using Caco-2 cells with HPLC detection.

**Results—**The apparent permeability coefficient ($P_{app}$) for RA and RA in *P. vulgaris* extracts was $0.2 \pm 0.05 \times 10^{-6}$ cm/s, significantly increased to $0.9 \pm 0.2 \times 10^{-6}$ cm/s after β-glucuronidase/sulfatase treatment. $P_{app}$ for UA and UA in *S. officinalis* extract was $2.7 \pm 0.3 \times 10^{-6}$ cm/s and $2.3 \pm 0.5 \times 10^{-6}$ cm/s before and after β-glucuronidase/sulfatase treatment, respectively. Neither compound was affected in permeability by the herbal extract matrix.

**Conclusion—**RA and UA in herbal extracts had similar uptake as that found using the pure compounds, which may simplify the prediction of compound efficacy, but the apparent lack of intestinal glucuronidation/sulfation of UA is likely to further enhance the bioavailability of that compound compared with RA.

### Keywords

*Prunella vulgaris*; *Salvia officinalis*; Rosmarinic acid; Ursolic acid; Permeability; Caco-2



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## 1. Introduction

Rosmarinic acid (RA, Fig. 1) is a caffeic acid (CA) derivative found in various botanicals, especially in *Prunella vulgaris*, a perennial herb known as self-heal used to treat sore throat, fever, and wounds (Psotová et al., 2003). RA and *P. vulgaris* limit liver damage derived from a model of bacterial inflammation (Osakabe et al., 2002) and inhibit nervous system inflammation in another model (Swarup et al., 2007). Ursolic acid (UA, Fig. 1), a pentacyclic triterpene acid, is also found in *P. vulgaris* but especially concentrated in sage leaves (*Salvia officinalis*), and inhibits inflammation-related changes in human gingival cells (Zdarilová et al., 2009) and in other models (Liu, 1995). This compound also has anti-mutagenic activity (Young et al., 1997). Both herbs have been used traditionally to treat inflammation in the mouth, and are of interest in inhibiting gastrointestinal inflammation which is relevant to colitis and colon cancer.

A major limiting step in the utilization of compounds from the diet such as RA and UA is their intestinal absorption and metabolism. Both compounds contain hydroxyls and are likely to be glucuronidated or sulfated in intestinal cells, forms that are generally considered to be less bioactive than parent compounds. Glucuronidation/sulfation has been demonstrated for RA but not for UA, and the plant matrix components might alter this metabolism but this has not been studied yet. The absorption or metabolism of RA has been examined *in vivo* to a limited extent (Baba et al., 2004; Konishi et al., 2005; Baba et al., 2005). When *Perilla* extract containing 200 mg of RA was orally administered to six men, RA in both plasma and urine was present predominantly as glucuronide and/or sulfate conjugated forms, at 0.6 ± 0.2% and 1.5 ± 0.4% of the total intake, respectively, within 48 h after ingestion (Baba et al., 2005). Gut microbes may metabolize RA to give phenolics such as caffeic acid (CA), *o*-coumaric acid (OCA) and m-hydroxyphenylpropionic acid, which are then absorbed by monocarboxylic acid transporter (MCT)-mediated active processes (Konishi and Kobayashi, 2005). After the oral administration of *Sambucus chinensis* ethanol extract (40g/kg to rats, containing 80 mg UA/kg) about 0.6% of ingested UA was recovered in plasma based on estimated blood volume and plasma area under curve of this compound, suggesting poor absorption or extensive metabolism and distribution to other body tissues (Liao et al., 2005). Both RA and UA as constituents of *P. vulgaris* may contribute to its bioactivities, and the effects of plant matrix on uptake of its key bioactive compounds are of interest.

Caco-2 cells are immortalized human epithelial colorectal adenocarcinoma cells and offer a standard rapid, reliable, and low-cost model for *in vitro* prediction of intestinal drug permeability and absorption (Hubatsch et al., 2007). Caffeic acid-related compounds, such as RA and chlorogenic acid, have been studied using Caco-2 cells and are proposed to transfer across the intestinal barrier by paracellular diffusion (Konishi and Kobayashi, 2005; Konishi and Kobayashi, 2004). The plant material matrix may alter absorption and bioavailability of phytochemicals (Manach et al., 2004). *P. vulgaris* and *S. officinalis* contain sugars, steroids, alkaloids, essential oils, flavonoids, polyphenols, triterpenoids, and saponins (Rasool et al., 2010; Cheung and Zhang, 2008; Lu and Foo, 2000; Loizzo et al., 2008). Therefore, it is crucial to investigate the effect that the plant matrix may have on the uptake of RA and UA found in *P. vulgaris* and *S. officinalis*. *In vitro* studies have not been done in association with the permeation of RA in *P. vulgaris* extracts using Caco-2 cells nor have different sources of *P. vulgaris* plant material been compared for their influence on RA uptake. Uptake of UA in the Caco-2 cell model also has not been investigated, either as a pure compound or from plant extracts. Our hypotheses were that the absorbability of RA and UA was independent of the plant extract matrix and this study was conducted to investigate the permeabilities of RA and UA as pure compounds and in *P. vulgaris* and *S. officinalis*

ethanol extracts across Caco-2 cell monolayers, to facilitate future studies of the efficacy of these herbs against gastrointestinal inflammation.

## 2. Materials and methods

### 2.1. Plant extraction

All *P. vulgaris* plant samples were provided by the U.S. Department of Agriculture North Central Regional Plant Introduction Station in Ames, IA. *S. officinalis* extract was provided by Sabinsa Corporation (Payson, UT). Seeds from accessions *P. vulgaris* Ames 27664, 27665 (both originally collected in North Carolina), and 27748 (collected in Missouri) were germinated in Petri plates at 25 °C (Voucher records: Herbarium specimen. Taken by: McCoy, J., USDA, ARS. On: 08/24/2006. Located at: ISC. Inventory sample: Ames 27664, 27665 and 27748. SD 04ncao01). The resulting seedlings, segregated by accession, were transferred to flats in a greenhouse (held at 20–25 °C). Upper flowering portions of 14-month-old plants were harvested at the time of peak flowering, dried, and ground. Four g aliquots of the ground samples were extracted with 500 mL of 95% ethanol by Soxhlet percolation for 6 h, filtered, dried by rotary evaporation and lyophilized. Then the extracts were redissolved in 0.5 mL of ethanol and stored at −20 °C under nitrogen. Information about the *P. vulgaris* accessions used for these experiments is available *via* the Germplasm Resources Information Network database at http://www.ars-grin.gov/npgs/acc/acc_queries.html.

### 2.2. HPLC analysis

RA (90%) and UA (92%), both from Sigma-Aldrich Co. (St. Louis, MO) and 2, 4, 4′-trihydroxybenzoin (THB, internal standard), synthesized in Dr. Hendrich's laboratory (Song et al., 1998) were dissolved in methanol to use them as standards. Methanol, acetonitrile (HPLC grade) and phosphoric acid (AR grade) were obtained from Fisher Scientific (Pittsburgh, PA).

HPLC analysis was performed on a Beckman Coulter 126 HPLC, equipped with photodiode array detector model 168 and a model 508 autosampler (Beckman Coulter, Inc., Brea, CA). The mobile phase was 1.25% phosphoric acid: acetonitrile (15% acetonitrile at 0 min, then increased to 84% at 15 min and held for 40 min; decreased to 15% at 65 min) at a flow rate of 0.5 ml/min. A reverse phase analytical YMC pack-ODS $C_{18}$ column (250 mm×4.6 mm × 5 μm, Waters Corp., Milford, MA ) was used at room temperature. The wavelength was 210 nm and the retention times were 21.5 min for RA and 26.5 min for UA. The limit of detection (LOD) and limit of quantitation (LOQ), defined as a signal/noise ratio ≥ 3 and ≥ 6, was 0.05 μM and 0.1 μM both for RA and UA. The linear regression equation for RA was $y = 95.256x - 0.0188$ with $R^2$ of 0.9995 and $y = 236.07x + 0.0014$ with $R^2$ of 0.9996 for UA, where y is the concentration of RA or UA, and x is the ratio of peak area of the standard to peak area of internal standard (THB). The intraday and interday CVs were 7.3 ± 2.5 % and 3.4 ± 1.6 % for RA, and 9.1 ± 3.8 % and 5.7 ± 2.1 % for UA across 0.1-100 μM.

### 2.3. Transepithelial transfer experiment

Caco-2 cells were obtained from American Type Culture Collection (Manassas, VA) at passage 18 and all experiments were performed from passages 25–30. The cells were cultured according to Hubatsch et al. (2007). Cytotoxicity of RA, UA, and extracts was measured according to Nasser et al. (2008). RA, UA, *P. vulgaris* ethanol extracts containing the same concentrations of RA, and *S. officinalis* extract containing the same concentrations of UA, at 1, 10, 20, 50, and 100 μM, were tested for cytotoxicity. DMSO in DMEM (Dulbecco's modified Eagle's medium, 0.3% v/v, Gibco Invitrogen, Carlsbad, CA) was used as control.

After the cells in the flask grew to 90–100% confluency, cells were trypsinized and seeded on collagen-coated polytetrafluroethylene membrane inserts (0.45 μm) fitted in bicameral chambers (Transwell-COL, 24 mm ID, Corning Inc., Corning, NY) at $1.2 \times 10^5$ cells/$cm^2$. The transepithelial electrical resistance (TEER) was tested by Millicell ERS meter (Fisher Sci., Pittsburgh, PA) to reflect the tightness of intercellular junctions and only cells with TEER ≥ 250 Ω·$cm^2$ were used for permeability study. At 14–16 d post seeding (90–100% confluence) on the transwell, RA, UA and extracts at non-cytotoxic concentrations or 100 μM of the paracellular marker, LY (lucifer yellow, Sigma-Aldrich Co., St. Louis, MO), dissolved in Hank's Buffered Salt Solution (HBSS, pH 7.4, Gibco Invitrogen, Carlsbad, CA), were added to the apical chamber, then basolateral solutions were collected after 0.5, 1, 2, and 4 h. After 4 h, apical solutions were collected and membrane on the transwell insert was placed in 1.5 mL of ice-cold sodium hydroxide (0.5 M) and sonicated with a probe-type sonic dismembrator (Biologics Inc., Manassas, VA); pH was adjusted to 7.0 and all samples were injected directly to HPLC for analysis. For LY quantification, the apical and basolateral solutions were transferred to a 96 well plate and read spectrophotometrically at 450 nm. Total cellular protein was determined by Coomassie (Bradford) assay (Pierce Laboratories, Rockford, IL).

### 2.4. Transepithelial transfer of single compounds and extracts after treating with β-glucuronidase/sulfatase

At 14–16 d post seeding of the cells on the transwell, 10 μM RA as a single compound and *P. vulgaris* ethanol extracts diluted to contain 10 μM RA or 20 μM UA and *S. officinalis* extract diluted to contain 20 μM UA were applied to Caco-2 cells. After collecting the basolateral solutions at 0.5, 1, 2, and 4 h as well as apical solutions and cell homogenates at the end, twenty μL of β-glucuronidase/sulfatase (Type H-2 from *Helix pomatia*, 85 units/L of glucuronidase and 7.5 units/L of sulfatase, Sigma-Aldrich Co., St. Louis, MO) were added and incubated overnight at 37 °C to release the parent compounds. These samples were then injected directly to HPLC.

Apparent permeability coefficients ($P_{app}$) were determined using the equation [10]: $P_{app} = (dQ / dt)\ (1 / (A \times C_0))$; dQ/dt was the permeability rate constant (μmol/s); A was the surface area of the membrane ($cm^2$); and $C_0$ was the initial concentration of the compound (μM). Basolateral recoveries (%) were calculated as the proportion of the original amount that permeated through the monolayer, which was calculated as the amount transported divided by the initial amount in the apical chamber. Transport rate (μM/h/$cm^2$) was calculated as the amount transported divided by incubation time and the area of the membrane.

### 2.5. Statistical analysis

Data are given as means ± S.D. Differences in cytotoxicity, $P_{app}$, transport kinetics, basolateral recoveries and transport rate of the pure compounds and extracts were evaluated statistically using ANOVA and Tukey's multiple comparison tests by SAS 9.1 (SAS Institute Inc., Cary, NC). Differences were considered significant at $p < 0.05$.

## 3. Results

### 3.1. Determination of RA and UA in P. vulgaris and S. officinalis ethanol extracts

The amounts of RA in ethanol extracts varied ~sixfold across the three *P. vulgaris* accessions; UA content in *P. vulgaris* extract was ~four- to twenty-fold less on a molar basis than was UA in *S. officinalis* extract (Table 1**)**. The ethanolic extract of *P. vulgaris* 27748 had threefold greater RA and tenfold more UA than the two other accessions studied. No RA was found in the *S. officinalis* extract.

### 3.2 Cytotoxicity test

For RA as a pure compound, concentrations greater than 50 μM were significantly cytotoxic compared with the control (0.3% v/v DMSO in DMEM, $p < 0.05$). Ethanolic extracts of *P. vulgaris* accessions containing > 20 μM RA showed significant cytotoxicity. Concentrations > 20 μM of UA as a pure compound and *S. officinalis* extract containing same amount of UA were toxic to the Caco-2 cells (data not shown). Therefore, 1, 2, 5, 10 μM of RA, and *P. vulgaris* ethanol extracts containing these concentrations of RA, and 2, 5, 10, 20 μM of UA and *S. officinalis* extract containing these amounts of UA were used for permeability studies.

### 3.3. Transepithelial transfer of single compounds and extracts before and after treating with β-glucuronidase/sulfatase

The basolateral recovery of paracellular marker, LY, was 0.5 ± 0.1%. In the basal chamber after 4 h, 1.3 ± 0.3, 1.2 ± 0.2, 1.4 ± 0.6 and 0.9 ± 0.4 % of parent RA was transferred for pure compound, or for RA in *P. vulgaris* 27664, 27665 and 27748 extracts, respectively (no significant differences, $p > 0.05$). The apical recoveries of RA were 86.7 ± 2.9, 85.8 ± 3.6, 81.2 ± 5.7 % and 79.1 ± 11.2 % for the pure compound, or for RA in *P. vulgaris* 27664, 27665 and 27748 extracts, respectively, with no significant differences ($p > 0.05$, data not shown).

The rate of membrane permeation was calculated for UA as a pure compound and from *S. officinalis* extract at the same doses. The uptake of UA increased linearly and significantly from 0.03 ± 0.01 to 0.2 ± 0.04 μM/h/cm$^2$ ($p < 0.01$) and was not saturable across the tested concentrations (5-20 μM, Fig. 2). It was also not saturable across tested time-points (0.5-4 h) both for pure compounds and UA contained in *S. officinalis* extract (Fig. 3A). Due to LOD of UA and the lesser amount of it in *P. vulgaris* extracts compared with RA, UA was not detected for apically-applied *P. vulgaris* extracts containing 1-10 μM RA. Basolateral recoveries of RA as pure compound and RA in *P. vulgaris* 27664 increased over time (RA in *P. vulgaris* 27665 and 27748 are not shown), although RA was not detected basolaterally at 0.5 and 1h before deconjugation (Fig. 3A). The basolateral transfer of UA was significantly greater than RA at each time point both for pure compounds or compounds contained in plant extracts ($p < 0.01$, Fig. 3A). No significant differences were found between RA as a pure compound and RA in *P. vulgaris* extracts or UA as a pure compound and UA in *S. officinalis* extract ($p > 0.05$) in apparent permeability coefficients ($P_{app}$, Table 2).

After deconjugation using β-glucuronidase/sulfatase, RA recoveries significantly increased from 1.3 ± 0.3% to 6.1 ± 1.4%, 1.2 ± 0.2% to 6.5 ± 2.7 %, 1.2 ± 0.03% to 4.9 ± 0.6 %, 1.1 ± 0.2% to 5.5 ± 1.2 % ($p < 0.01$) in basal chamber for the single compound, or for RA in *P. vulgaris* 27664, 27665 and 27748 extracts, respectively (Fig. 3C, RA in *P. vulgaris* 27665 and 27748 are not shown). The transfer of RA glucuronide/sulfate conjugates was not saturable during 4 h and increased linearly with the incubation time ($p < 0.01$, Fig. 3B). To check the mass balance, the total recoveries (apical + basolateral) of RA increased from 80-88% before deconjugation to 84-93% after deconjugation for pure compound and RA in extracts, with no new peaks detected. The basolateral recoveries of UA were 19.0 ± 4.2% or 15.9 ± 3.2% for pure compound before or after incubation with β-glucuronidase/sulfatase, and 17.6 ± 2.5% or 14.5 ± 4.3% for *S. officinalis* extract, not statistically different ($p > 0.05$, Fig. 3C). No significant differences were found in the apical recoveries of RA or UA ($p > 0.05$) and neither RA nor UA was found in cells before or after deconjugation. $P_{app}$ for UA or UA in *S. officinalis* extract did not change after deconjugation reaction (Table 2**)**. Both RA as a single compound and RA in P. vulgaris extracts showed the same increase in $P_{app}$ after deconjugation (Table 2).

## 4. Discussion

Because *in vivo* absorption studies performed with laboratory animals and humans are expensive and time consuming and can pose ethical challenges, Caco-2 cells are a useful alternative to study uptake and transport of important compounds from plant materials (Walgren et al., 1998). Plant matrices may influence phytochemicals by altering transporters of some compounds or of compound metabolites (Mukinda et al., 2010). Phytochemical biotransformation may also be altered by other components of the plant matrix. To establish the effect that the plant matrix had on the *in vitro* uptake of RA and UA found in *P. vulgaris* and *S. officinalis*, two herbs that may be important for gut health, the uptake of pure solutions of the two compounds and extracts of two herbs that are major sources of these compounds were compared in Caco-2 cell system.

RA in *P. vulgaris* and UA in *S. officinalis* extracts had similarly efficient absorption as that in pure solutions of each compound in Caco-2 cells (Fig. 3A and Table 2, $p > 0.05$); this was not influenced by varied contents of RA across three accessions collected from different sources. The $P_{app}$ for RA in *Salvia miltiorrhiza* was $0.5 \pm 0.3 \times 10^{-6}$ cm/s in Caco-2 cells (Lu et al., 2008), consistent with our results. This result is also consistent with *in vivo* studies showing poor absorption of intact RA and suggesting that the absorption of RA is independent of other constituents contained in plant extracts (Baba et al., 2004). The impact of plant matrices on the bioavailability of phytochemicals is highly dependent on the compound of interest. For example, the absorption of epicatechin was not influenced by the ingredient composition of beverage food matrices *in vitro* (Neilson et al., 2009), but *Hypericum perforatum L.* product matrices affected the transport of rutin, hyperoside and isoquercitrin across Caco-2 cells (Gao et al., 2010), probably due to differences in matrix phytochemical composition and transport characteristics, i.e. paracellular transfer, carrier mediated or active transport. In our study, both RA and UA were major components of their respective plant material (~3% or greater by weight, and ~ 5-50 mmol/L of ethanolic plant extract). It could be useful to compare RA or UA uptake from plant matrices in which these components were less concentrated. Studies are also needed to investigate the influences of preservation or processing of plant extract on the bioavailability of RA and UA both *in vitro* and *in vivo*.

If the basolateral movement of the paracellular marker, LY, is less than 0.7% and TEER value is greater than 250 $\Omega \cdot cm^2$, the established monolayer is considered to be tight enough for permeability experiments (Ohashi et al., 2009). In our permeability studies, we tested the basolateral recoveries at time points and interval times chosen for a hydrophilic paracellularly transferred compound (RA) (Hubatsch et al., 2007). RA uptake was not saturable during 4 h incubation period (Fig. 3A), indicating that passive diffusion occurred for this parent compound. We only measured the uptake of RA at 10 μM due to its cytotoxicity; RA was not detected at 0.5 and 1 h because of its LOD and the low concentration applied to the apical chamber. Unidirectional transport (apical to basolateral transfer) was investigated for RA in our study and the pH of both apical and basolateral solutions was 7.4 (to simulate the environment of small intestine), because Konishi and Kobayashi (2005) showed that permeation of RA from the apical to the basolateral side is similar to that from basolateral to apical side; the uptake in the presence of a proton gradient (pH gradient 6.0/7.4) was nearly the same as that in the absence of a proton gradient (pH gradient 7.4/.4), implying that proton coupled polarized transport was not involved for RA. The transepithelial flux of RA was inversely correlated with TEER in our experiment (data not shown), consistent with restriction of intestinal absorption of RA when the epithelial tight junction is tight enough, a finding similar to that shown for another caffeic acid derivative, chlorogenic acid (Konishi and Kobayashi, 2004).

Our finding of increased basolateral RA recoveries after deconjugation (Fig. 3C) is the first direct report of this type of intestinal biotransformation of RA; glucuronide/sulfate conjugates were excreted to the basolateral side (toward the circulation) rather than apical side (the intestinal lumen), but this finding is consistent with *in vivo* observations of RA glucuronide/sulfate conjugates as major forms of this compound in rat and human plasma (Konishi et al., 2005; Baba et al., 2005). These results suggest that RA is absorbed *via* both paracellular and transcellular diffusion and MRP or OATP (organic anion transporter protein) transporters might be involved in the permeation of glucuronidated or sulfated RA, because anionic conjugates (glutathione, glucuronide or sulfate) cannot exit cells unless an MRP transporter is present (Peng et al. 1999). Intestinal biotransformation of RA seemingly differs from that of its possible metabolites, glucuronide/sulfate conjugates of caffeic acid, ferulic acid and *o*-coumaric acid which were found only apically (Kern et al., 2003). This might be due to the specificity of MRP transporters to the glucuronide/sulfate conjugates of different caffeic acid derivatives and the locations of various MRP in apical or basolateral sides of the enterocytes (Peng et al., 1999).

The plant extracts did not affect transfer of either UA or RA, and likewise the pure compounds and the compounds from the plant extracts behaved similarly in their apparent biotransformation as well (Fig. 3A, B and C and Table 2), establishing that the transfer of RA and UA was independent of the of the plant extract matrix for the two herbs studied. The transfer of UA seemed simpler than that observed for RA. The rate of membrane permeation of UA increased linearly with concentration and was not saturable at the tested time-points (**Fig**. 2 and 3A), indicating uptake by passive diffusion. Because the basolateral transfer of UA was greater than RA at each time point (Fig. 3A), and the partition coefficient (Log P) of RA was 0.2, compared with Log P of UA of 6.4 (Konishi et al. 2005; Bérangère et al., 2004), more hydrophobic UA was more absorbable than RA. This result is consistent with an interpretation of previous *in vivo* findings in rats that the low plasma concentration of UA was not due to poor absorption but to extensive uptake of UA by other tissues (Liao et al. 2005). UA was absorbed and transferred by Caco-2 cells apparently with little glucuronidation/sulfation, which is likely to further enhance the bioavailability of UA compared with RA (Fig. 3C and Table 2). This lack of UA biotransformation might be due to the differences in glucuronidation and sulfation of aliphatic alcohol (UA) and phenol (RA) alcohol or because of the distinct structures of the two compounds: triterpenoid (UA) vs. caffeic acid derivative (RA). But two studies showed that there was wide variability in affinity for UDP-glucuronosyltransferase (UGT) and sulfotransferase (ST) across both general classes of substrates (Chen et al., 1996; Ebner and Burchell, 1993). One related triterpenoid, glycyrrhetinic acid (50-300 μM), was found to inhibit UGT2B7 activity in human liver microsomes (Nakagawa et al., 2009), which might also occur for UA. To our knowledge, no other *in vivo* or *in vitro* study has been done on the metabolism of ursolic acid. It is possible that UA may undergo additional glucuronide or sulfate conjugation in the liver. Future studies are required to elucidate the structures of RA and UA metabolites, which affect compound transfer mechanism and efficacy.

In conclusion, RA was transferred across Caco-2 cells almost entirely in conjugated form, but UA was absorbed and transferred mostly intact, both independent of extract matrix. Our results predict no effect of plant matrix on the efficacy of either compound. It will be important to measure the uptake of gut microbial metabolites of RA and effects of RA on the expression of efflux transporters and tight junction proteins which would particularly affect a compound transferred paracellularly such as RA. Cellular models of oral as well as intestinal uptake, metabolism and anti-inflammatory activity of RA and *P. vulgaris* and UA and *S. officinalis* will be of interest as well, given traditional uses of these herbs.

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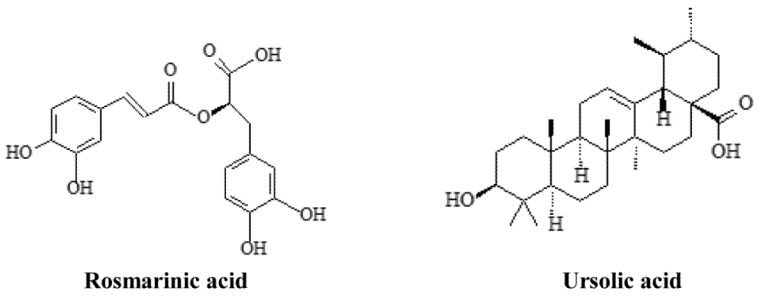


**Fig. 1.**
The chemical structures of rosmarinic and ursolic acids.

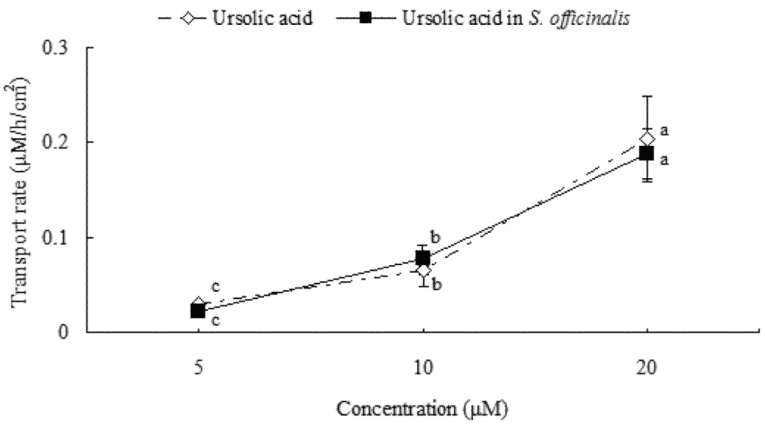


**Fig. 2.**

The percent transported of ursolic acid for pure compound and *S. officinalis* extract across Caco-2 cell monolayer as a function of concentration at 5–20 μM after 4 h uptake study. *S. officinalis* extract was diluted to apply the same concentrations to the cells as were used for the pure compounds. Data are the mean ± S.D (n=9). Means bearing different letters were significantly different ($p < 0.01$).

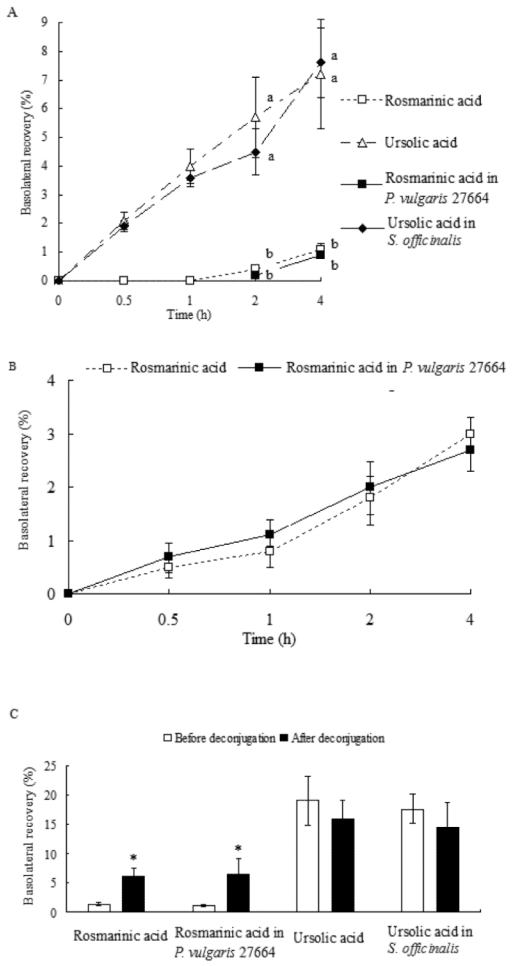


**Fig. 3.**

Characteristics of transfer of rosmarinic (RA) and ursolic acids (UA) for pure compounds or plant extracts across Caco-2 cells before and after deconjugation of post-experimental basolateral solutions. A. Before deconjugation. No RA was found at 0.5 or 1 h. Means bearing different letters were significantly different ($p < 0.01$). B. After deconjugation with β-glucuronidase/sulfatase; for RA and RA in *P. vulgaris* 27664. C. Total basolateral recovery of RA and UA after deconjugation over 4 h incubation. * The basolateral recoveries of RA after β-glucuronidase/sulfatase incubation were significantly different from that before the enzyme treatment ($p < 0.01$). Basolateral recoveries were calculated as the amount transported divided by the initial amount in the apical chamber during 4 h uptake study. Data are the mean ± S.D (n=9).

## Table 1

The contents of rosmarinic and ursolic acids in ethanol extracts of *P. vulgaris* and *S. officinalis* determined by HPLC.

| | RA g/L (mM) | UA g/L (mM) |
|---|---|---|
| *P. vulgaris* 27664 (174.4 g/L ) | 8.4 ± 0.6 (23.3 ± 1.7) | 0.1 ± 0.0 (0.2 ± 0.0) |
| *P. vulgaris* 27665 (66.2 g/L ) | 3.2 ± 0.3 (9.0 ± 0.8) | 0.1 ± 0.0 (0.1 ± 0.0) |
| *P. vulgaris* 27748 (117.0 g/L ) | 19.9 ± 1.1 (55.2 ± 3.1) | 0.6 ± 0.1 (1.0 ± 0.2) |
| *S. officinalis* (10 g/L ) | -- * | 2.6 ± 0.4 (5.8 ± 0.9) |

* No RA was found in *S. officinalis* extract.

## Table 2

Apparent permeability coefficients ($P_{app}$) for lucifer yellow, rosmarinic acid, ursolic acid and extracts across the Caco-2 monolayer.

| | **$P_{app}$ (cm/s $\times 10^{-6}$) before deconjugation** | **$P_{app}$ (cm/s $\times 10^{-6}$) after deconjugation [c]** |
|---|---|---|
| LY | 0.3 ± 0.1 | -- |
| RA[a] | 0.2 ± 0.0 | 0.9 ± 0.2$^{\dagger}$ |
| RA in *P. vulgaris* 27664 | 0.2 ± 0.0 | 1.0± 0.4$^{\dagger}$ |
| RA in *P. vulgaris* 27665 | 0.2 ± 0.1 | 0.7 ± 0.1$^{\dagger}$ |
| RA in *P. vulgaris* 27748 | 0.1± 0.1 | 0.9± 0.1$^{\dagger}$ |
| UA[b] | 2.8 ± 0.1$^{*}$ | 2.4 ± 0.4$^{*}$ |
| UA in *S. officinalis* | 2.5 ± 0.4$^{*}$ | 2.2 ± 0.6$^{*}$ |

--: not determined

[a] RA was 10 μM for pure compound and *P. vulgaris* extracts containing the same content of RA.

[b] $P_{app}$ of UA as a pure compound and in *S. officinalis* extract was calculated using non-toxic concentrations (5, 10 and 20 μM).

[c] The $P_{app}$ of rosmarinic and ursolic acids after treating with $\beta$-glucuronidase/sulfatase.

$^{*}$ Significantly greater compared with $P_{app}$ of LY and RA ($p < 0.01$).

$^{\dagger}$ Significantly different before and after deconjugation ($p < 0.01$).