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Ingestion of Oregano Extract Increases Excretion of Urinary Phenolic Metabolites in Humans

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Despite the promising antioxidant action of Lamiaceae herbs *in vitro*, human studies on these potential sources of dietary antioxidants have remained scarce. In this work, the phenolic acids recovered in human urine after single ingestion of *Origanum onites* extract were analyzed. The excretion was increased 4- and 2-fold during 0−24 and 24−48 h of the follow-up, respectively. The mean increase in the excretion of phenolic compounds exceeded the ingested amount of identified phenolic acids. The result can be partly explained by rosmarinic acid, the main identified phenolic constituent in the extract, as well as flavonoids present in minor amounts, presumably being metabolized into a double amount of simple phenolic metabolites. Furthermore, unidentified phenolic constituents in the extract partly contribute to the excretory increase. The main metabolite, *p*-hydroxybenzoic acid, was excreted rapidly. The results show that constituents of oregano extract and, in particular, their metabolites may contribute to the dietary intake of phenolic antioxidants.

KEYWORDS: Antioxidants; Lamiaceae; metabolism; oregano; *Origanum onites*; phenolic acids; rosmarinic acid; urine

INTRODUCTION

The interest and research focused on the bioavailability and metabolism of phenolic substances in leafy spices is well-justified. Lamiaceae herbs are rich in various phenolic compounds (*1*, *2*) and characterized by the occurrence of rosmarinic acid (*3*, *4*). Rosmarinic acid alone has been ascribed to a number of bioactivities (*5*). Because of the antioxidant activity of these herbs in laboratory test models (*4*, *6−11*), they have been suggested to have beneficial effects on human health. The contribution of several culinary and medicinal herbs to the total intake of dietary antioxidants has been assessed (*12*), and they were reported to be a significant dietary antioxidant source, even superior to many other food groups (*13*). Selected representatives (oregano, sage, thyme, and peppermint) of the Lamiaceae family involved in the study were found to contain antioxidant constituents in amounts sufficient to make a significant contribution to the dietary intake of phenolic antioxidants. The antioxidant hypothesis is supported by epidemiological data, according to which an increase in the consumption of polyphenol-rich constituents to the diet is associated with a decrease in the incidence of oxidative-stress-related diseases such as cardiovascular diseases, cancers, and neurodegenerative diseases (*14*, *15*). In the above-mentioned reviews, the proposed mechanisms of action against cardiovascular diseases including the inhibition of low-density lipoprotein oxidation, inhibition of platelet aggregation, and adhesion and improvement of endothelial dysfunction have been discussed. Despite the epidemiological evidence and promising results from different laboratory assays, to evaluate the potential beneficial action of herb antioxidants *in vivo*, studies clarifying the bioavailability, metabolism, and excretion of phenolic substances found in herbs are needed.

Some studies on the absorption and/or metabolism of rosmarinic acid or *P. frutescens* herb (belonging to the Lamiaceae family) extract containing rosmarinic acid *in vitro* (*16*) in rats (*17−21*) and in humans (*18*, *22*) have been published. In the work presented herein, the absorption and metabolism of phenolic compounds from dried aqueous extract of *Origanum onites* L. (Turkish oregano, pot marjoram, or white marjoram) was studied in healthy humans based on the analysis of urinary excretion of 13 different phenolic acids prior to and after a single ingestion of the extract.

MATERIALS AND METHODS

Preparation of the *O. onites* Extract. A total of 100 g of the dried and cut herb material (originated from Turkey, supplied by Control-Ox Ltd., Helsinki, Finland) was placed in a 2000 mL round-bottom flask, and 1000 mL of water was added. The flask was connected to a Ph. Eur. hydrodistillation apparatus (described in European Pharmacopoeia, 5th ed.), and the water was boiled for 2 h. The water from the

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flask was removed; 600 mL of fresh water was added; and the mixture was boiled again for 1 h. Water fractions were combined, filtered through qualitative number 4 filter paper (Whatman International Ltd., Maidstone, U.K.), freeze-dried, and stored at 4 °C. The yield of the dry extract from the original plant material was found to be about 27%. For the experiment, the extract was packed in hard-gelatin capsules using a manual encapsulating device.

Determination of the Total Phenolic Content of the Extract. The total phenolic content of the extract was determined as gallic acid equivalents using the Folin-Ciocalteu method (*23*) as described earlier (*9*).

Study Subjects, Diet, Extract Supplement, and Sample Collection. Six healthy (no chronic diseases, body mass index <32 kg/m^2) subjects (four females and two males) aged 28−38 years received a single oral dose (3.75 g) of *O. onites* extract packed in hard-gelatin capsules. For 7 days prior to the experiment, the subjects followed a washout diet and refrained from consuming phenolic-rich foods and beverages. The washout diet consisted mainly of dairy products, meat, fish, chicken, potato, pasta, rice, and white bread. The same diet was continued for the 48 h follow-up time. To maintain a steady water balance, the study subjects were informed to continue their normal water drinking habits and to avoid hard physical exercise during the study. To determine the baseline urinary excretion of phenolic acids, 24 h urine was collected before the experiment. To evaluate the absorption, metabolism, and excretion of the phenolic compounds from the extract, total urine production were collected 2, 4, 6, 8, 12, 24, 30, 36, and 48 h after the ingestion of the extract. The single urine collections (0−2, 2−4 h, etc.) are later on referred to as single-spot urine samples. The volume of the collected urine samples was determined, and aliquots of 2 × 10 mL were stored at −70 °C until analyzed.

Standards. Oregano extract was screened for the presence of 46 phenolic compounds: 2,3-dihydroxy-, 2,4-dihydroxy-, 2,5-dihydroxy-, 2,6-dihydroxy-, and 3,5-dihydroxybenzoic acids, protocatechuic acid, *m*-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, resorcinol, rosmarinic acid, vanillic acid, catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, apigenin, apigenin-7-*O*-β-glucoside, cosmosin, eriodictyol, ficetin, hesperetin, isorhamnetin, isovitexin, kaempferol, luteolin, luteolin-7-*O*-glucoside, myricetin, naringenin, naringin, quercetin, rhamnetin, rutin, and vitexin. On the basis of the phenolic acid composition of the extract, the following 13 phenolic acids were analyzed from urine samples: 3,4-dihydroxyphenylacetic acid, *m*-hydroxyphenylacetic acid, *m*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, rosmarinic acid, ferulic acid, gallic acid, protocatechuic acid, syringic acid, and vanillic acid. The purity of the standards purchased from usual suppliers was 98% or higher, and all cinnamic acid derivatives were of the trans conformation.

Analysis of Phenolic Compounds in *O. onites* Extract and Urine. Phenolic compounds in *O. onites* extract and urine samples were analyzed as described earlier (*24*). In brief, the analyses were carried out with high-performance liquid chromatography (HPLC) using a coulometric electrode array detector (CEAD) (ESA, Inc., Chelmsford, MA). Different potential was set on each channel of the detector to oxidize analytes under interest. The mobile phase consisted of two eluents: (A) 50 mM KH_2PO_4/H_3PO_4 buffer (pH 2.3)/MeOH (90:10, v/v) and (B) 50 mM KH_2PO_4/H_3PO_4 buffer (pH 2.3)/MeOH/ACN (40:40:20, v/v/v). A flow rate of 0.3 mL/min and injection volume of 10 μL were employed in both analyses. Analytical column Inertsil ODS-3 (GL Sciences, Inc., Japan) 150 × 3 mm packed with 3 μm end-caped particles and guard column Quick Release C18 (Upchurch Scientific, Inc., WA) 10 × 3 mm packed with 5 μm particles were used. Limits of detection (LOD) and linear ranges for signals were determined to evaluate the suitability of the detection conditions for phenolic acids in urine and in the *O. onites* extract. Phenolic acids in the *O. onites* extract were quantified after the combination of enzymatic (*Helix pomatia* juice) and base hydrolysis. In the enzymatic hydrolysis, ascorbic acid was added to the sample, followed by a hydrolysis reagent containing 2500 units/mL of β-glucuronidase from *H. pomatia* in 0.3 M sodium acetate buffer at pH 4.1. Sample was incubated at 60 °C for 2 h. Into a cool sample, MeOH was added; the sample was shaken vigorously and centrifuged. The supernatant was diluted up to volume with MeOH. The stability of rosmarinic acid during the enzymatic hydrolysis and at pH 2 at 37 °C (stomach) was separately confirmed, and no loss was observed. Urinary phenolic acids were quantified after the β-glucuronidase−sulfatase enzyme hydrolysis and diethyl ether extraction. Flavonoids were screened after the enzymatic hydrolysis by using the same HPLC conditions as for phenolic acids, and catechins were determined as described earlier (*25*). The overall performance of the urine sample pretreatment was followed by the use of a control sample, which was included into each batch of 20 samples. Interassay coefficients of variation for the analytes ranged from 6.4% (ferulic acid) to 15% (protocatechuic acid). Recovery of the analytes ranged from 79% (3,4-dihydroxyphenylacetic acid) to 91% (*p*-hydroxybenzoic acid). Quantitative results for urinary phenolic acids were obtained after the recovery correction.

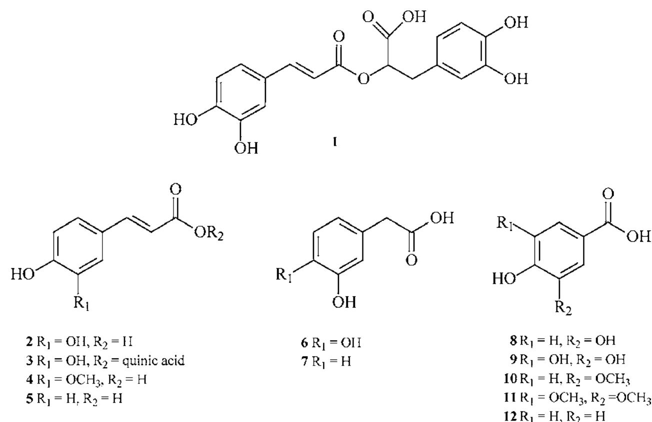

Figure 1. Phenolic acids in the *O. onites* extract and/or urine. **1**, rosmarinic acid; **2**, caffeic acid; **3**, chlorogenic acid; **4**, ferulic acid; **5**, *p*-coumaric acid; **6**, 3,4-dihydroxyphenylacetic acid; **7**, *m*-hydroxyphenylacetic acid; **8**, protocatechuic acid; **9**, gallic acid; **10**, vanillic acid; **11**, syringic acid; and **12**, *p*-hydroxybenzoic acid.

The structures of the phenolic acids detected in the extract and/or urine are presented in **Figure 1**.

RESULTS

Total Phenols, Phenolic Acids, and Flavonoids in *O. onites* Extract. The total phenolic content of the extract was determined to be 13.4% of the dry matter, determined as gallic acid equivalents (GAE). The single oral dose of oregano extract was chosen to be 3.75 g, with the calculated amount of total phenolic compounds per dose thus being about 500 mg of GAE. In the HPLC analyses, the extract was found to contain rosmarinic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, and gallic acid, altogether 16.8 mg/g, of which 75% was rosmarinic acid and 12% was protocatechuic acid. The supplemented amount of the extract was 3.75 g, containing 63 mg (223 μmol) of quantified phenolic acids. This amount represented 12.6% of the amount of total phenolic compounds expressed as GAE. Of the screened flavonoids, luteolin (3.6 mg/g) and eriodictyol (0.19 mg/g) were found in the extract altogether corresponding to 49 μmol of flavonoids in the supplemented dose of the extract. The amounts of total phenols and identified phenolic acids in the extract and in the supplemented dose are presented in **Table 1**.

Phenolic Acids and Their Metabolites in Human Urine. The daily excretion of each subject for each phenolic acid prior to and following the supplementation is presented in **Figure 2**. The baseline excretion of the identified phenolic compounds during the preceding 24 h was 95 ± 11 μmol/day. The major

Table 1. Excreted Amounts of Different Phenolic Acids before and after the *O. onites* Extract Supplementation

compound	baseline[a]	mg/g in extract	supplemented mg (μmol)	excreted 1[b]	2	3	4	5	6	mean ± SD
rosmarinic acid		12.7	47.7 (133)							
gallic acid		0.15	0.56 (3)							
chlorogenic acid		0.31	1.2 (3)							
protocatechuic acid	2	2.1	7.8 (51)	22	37	24	24	34	33	29 ± 6
caffeic acid	1			3	8	13	10	6	7	8 ± 3
ferulic acid	11	0.50	1.9 (10)	23	34	40	56	45	58	43 ± 13
syringic acid	1			9	6	8	11	9	12	9 ± 2
vanillic acid	32			64	229	55	55	130	36	95 ± 73
p-hydroxybenzoic acid	21			176	258	212	221	260	415	257 ± 83
p-coumaric acid	1	1.0	3.8 (23)	13	8	3	4	3	1	5 ± 4
3,4-dihydroxyphenylacetic acid	9			21	35	25	32	32	41	31 ± 7
m-hydroxyphenylacetic acid	16			131	44	57	102	78	175	98 ± 49
total	95		62.9 (223)	462	659	437	515	597	778	575

[a] Mean value during the preceding 24 h ($n = 6$). [b] Numbers refer to the study subjects, and values (in micromoles) present total excretion during the 48 h follow-up time.

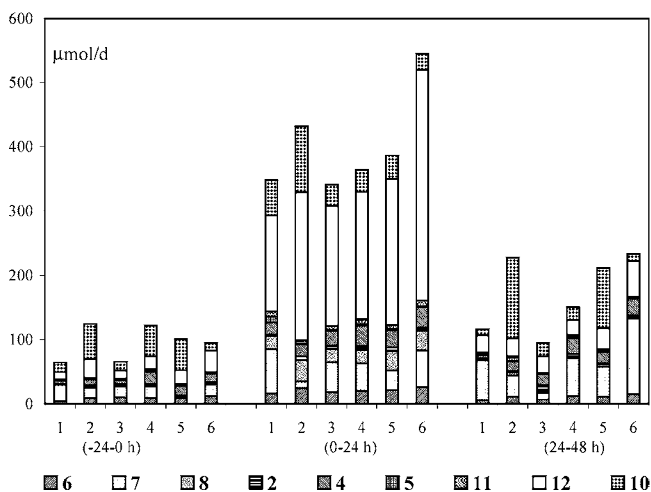

Figure 2. Mean excretion of the phenolic acids and their metabolites before and after the single oral dose of the extract. Numbers 1–6 refer to the study subjects. Numbering of compounds refers to the caption of **Figure 1**.

compound excreted in urine prior to the supplement was vanillic acid, followed by *p*-hydroxybenzoic acid, *m*-hydroxyphenylacetic acid, ferulic acid, and 3,4-dihydroxyphenylacetic acid, respectively. Low amounts of protocatechuic acid, syringic acid, *p*-coumaric acid, and caffeic acid were also detected in urine.

During the first 24 h after the supplementation, the mean excretion of the identified phenolic acids was 403 ± 69 μmol/day, and during the next 24 h, the mean excretion of the identified phenolic acids was 172 ± 21 μmol/day. During the first day of the follow-up, the main compound excreted was *p*-hydroxybenzoic acid, followed by vanillic acid, *m*-hydroxyphenylacetic acid, protocatechuic acid, ferulic acid, and 3,4-dihydroxyphenylacetic acid. Minor amounts of syringic acid, caffeic acid, and *p*-coumaric acid were also detected in urine. During the second day of the follow-up, *m*-hydroxyphenylacetic acid was the main compound excreted in urine, followed by vanillic acid, *p*-hydroxybenzoic acid, ferulic acid, and 3,4-dihydroxyphenylacetic acid. Amounts of protocatechuic acid, caffeic acid, *p*-coumaric acid, and syringic acid excreted were low. Rosmarinic acid, gallic acid, or chlorogenic acid were not detected in any urine samples before or after the supplementation.

During the total 48 h follow-up time, the mean increasing effect of the supplement on the excretion of identified phenolic compounds (baseline extraction subtracted) was altogether 385 ± 114 μmol (**Table 1**). The mean urinary excretion of each phenolic acid prior to and following the supplementation is presented in **Figure 3**, and the excretion profiles (all compounds summarized) for the individual study subjects are shown in **Figure 4**. Some possible metabolic reactions of the oregano extract constituents are suggested in **Figure 5**.

DISCUSSION

A high proportion of components in the plant material were extractable with hot water, as can be deduced by the high yield of the dry extract (27%). The total phenolic content of the extract was found to be rather high (134 mg of GAE/g). Even though the extract was screened for 46 phenolic compounds, the phenolic composition was only partly defined in the analyses, and this fact, in addition to the analytical differences between the total phenol analysis and HPLC analyses for individual compounds, explains the difference between the total phenol results and results of individual component analyses.

The main identified phenolic constituent in the extract was rosmarinic acid, representing 75% of the identified phenolic acids. Other phenolic acids: protocatechuic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, and gallic acid, were present in the extract in notably lower amounts. The extract also contained minor amounts of flavonoids luteolin and eriodictyol. The amount of phenolic compounds provided in the supplement in our study is comparable with most of the other studies, because the supplement provided about 500 mg of GAE total phenols and 63 mg (223 μmol) of identified and quantified phenolic acids. The estimates of the intake of dietary polyphenols and the amounts of phenolic compounds provided in study supplements range from tens of milligrams to nearly 1000 mg/day, depending upon the class of phenolic compounds in question (*26*).

By the baseline urine collection period, the volunteers had consumed a washout diet with strictly restricted intake of phenolic compounds for 1 week and the interindividual variation in the baseline excretion was low. The major metabolites excreted were vanillic acid, *p*-hydroxybenzoic acid, *m*-hydroxyphenylacetic acid, ferulic acid, and 3,4-dihydroxyphenylacetic acid.

After ingestion of *O. onites* extract, urinary excretion of the identified phenolic compounds was increased remarkably (**Figure 2**). The mean baseline excretion of the identified phenolic compounds was 95 μmol/day, and during the 2 days of follow-up, the mean baseline excretion of the identified phenolic compounds was 403 and 172 μmol/day, respectively. The

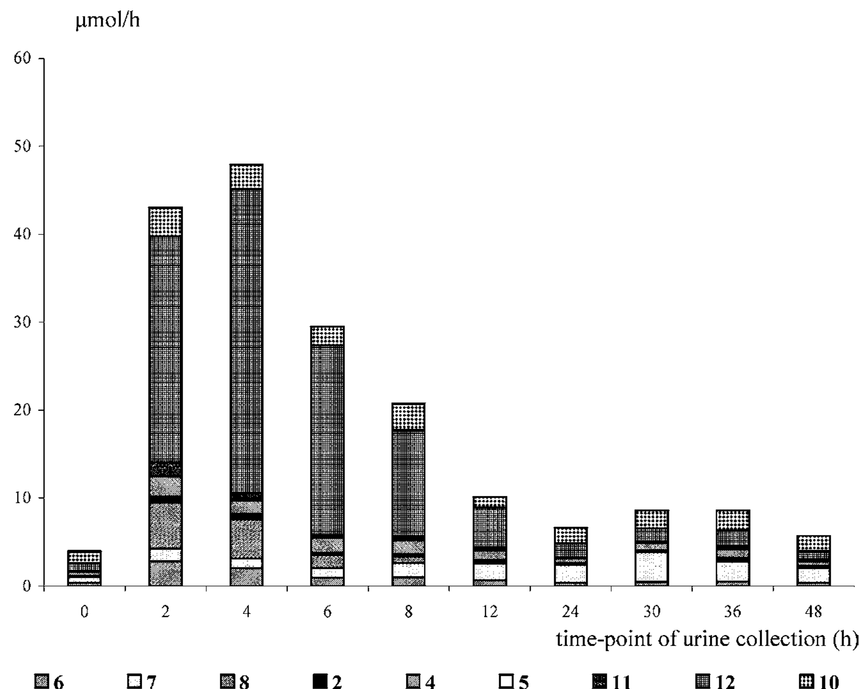

Figure 3. Mean urinary excretion (μmol/h) of the different phenolic acids during the 48 h follow-up time. Time-point "0" represents the hourly excretion at the baseline. Numbering of compounds refers to the caption of **Figure 1**.

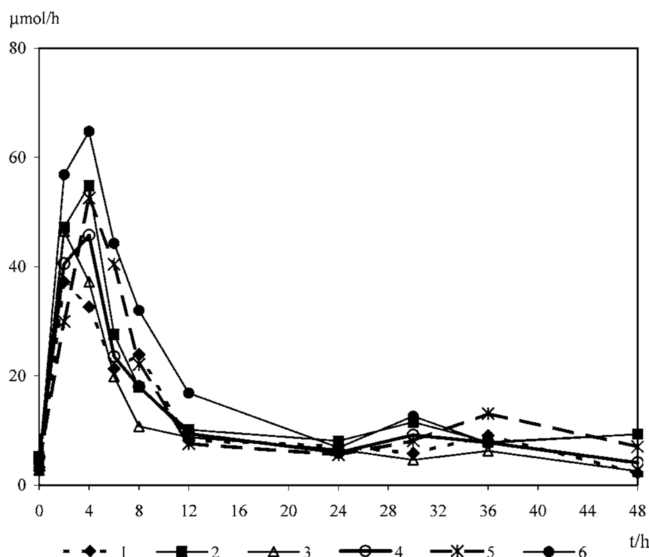

Figure 4. Urinary excretion (μmol/h, all compounds summarized) for the phenolic compounds and their metabolites during the 48 h follow-up time. Numbers 1–6 refer to the study subjects.

maximum excretory peak for all of the phenolic compounds summarized together was observed between 2−4 h postsupplementation (**Figure 4**), because of the early excretory maximum of the major compound *p*-hydroxybenzoic acid (**Figure 3**). Even though individual differences were found in excretion of the separate compounds (**Table 1**), the pattern of total excretion in all study subjects was rather similar (**Figure 4**). Of the 223 μmol of originally identified phenolic acids in the dose of the extract, 49 μmol of the following compounds were excreted during the follow-up time (baseline excretion subtracted): protocatechuic acid, ferulic acid, and *p*-coumaric acid. About half of ingested protocatechuic acid was excreted as such, whereas ferulic acid was excreted in a higher amount than that ingested, suggesting that it is at least partly derived from the metabolism of other phenolic acids. Only a small amount of *p*-coumaric acid ingested ended up in urine.

During the 48 h follow-up time, the excretion of identified phenolic compounds and their metabolites increased altogether by 385 μmol as compared to the baseline excretion, suggesting that the absorbed phenolic constituents of oregano extract were intensively metabolized prior to urinary excretion. The increase in the mean molar excretion of the identified phenolic acids and their metabolites was more than the molar supplemented amount, an observation which could be partly explained by cleavage of the ester structure of rosmarinic acid into caffeic acid and lactic acid residues (*17*). The metabolism that occurred may also explain why intact rosmarinic acid was not detected in urine. The flavonoids luteolin and eriodictyol found in the extract also possess a structure yielding 2 mol of metabolites/1 mol of the parent compound after the cleavage of the heterocyclic ring (*27*). Another explanation for the urinary recovery being more than the supplemented amount of phenolic constituents is the unidentified phenolic compounds present in the extract. These possible metabolic reactions of the oregano extract constituents are suggested in **Figure 5**.

Neither free nor conjugated rosmarinic acid was detected in any single-spot urine sample. To ensure that this was not due to the enzymatic *H. pomatia* hydrolysis of urine, the stability of rosmarinic acid during the enzymatic hydrolysis was confirmed. Earlier, the absorption of rosmarinic acid has been studied *in vitro* in Caco-2 cells and the absorption by paracellular diffusion from this model of small intestine epithelium was reported to be very low (*16*). The absorption characteristics of rosmarinic acid, an ester of caffeic and 3-(3,4-dihydroxyphenyl)-lactic acids, in Caco-2 cells resembled those of chlorogenic acid, an ester of caffeic and quinic acids (*28*). Caffeic acid was found to be absorbed 10 times more effectively than chlorogenic acid. The difference in absorption characteristics between chlorogenic and caffeic acids has also been shown in rats (*29*). Phenolic acids like chlorogenic acid are stable in acidic conditions such as in the stomach (*30*), similar to rosmarinic acid in our study.

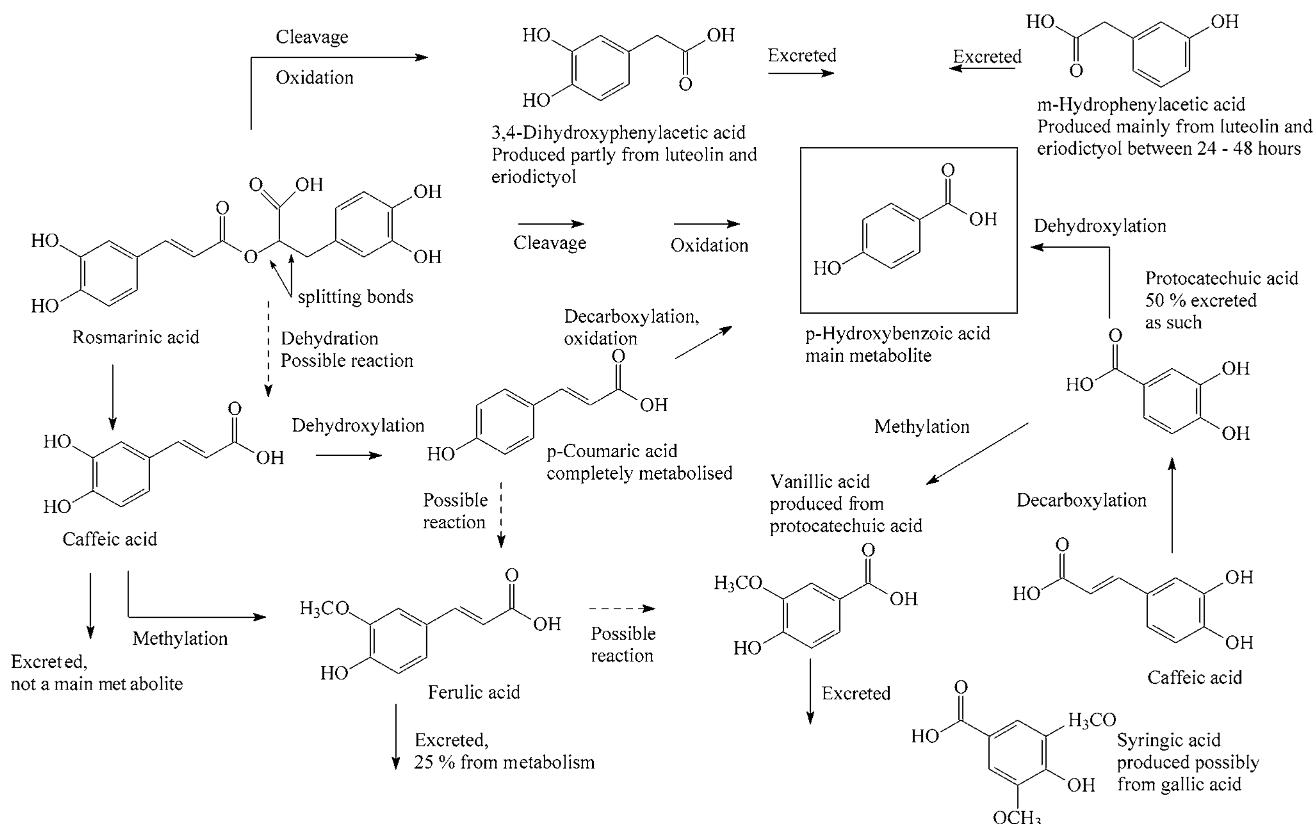

Figure 5. Possible metabolic reactions of constituents of oregano extract in humans.

Caco-2 cells were reported to be capable of metabolizing esters of hydroxycinnamates, and evidence for sulfation, glucuronidation, and methylation of these compounds by Caco-2 cells was provided (*31*). However, later on, it was reported that rosmarinic acid appeared to be unsusceptible to hydrolysis by mucosa esterase in Caco-2 cells (*16*). On the basis of studies carried out with chlorogenic acid, esterified hydroxycinnamates are claimed not to be cleaved in the gastric lumen (*32*, *33*) or in the small intestine but in the colon by the esterase activity of the gut microflora (*34*). However, in one human study (*35*), 33% of ingested chlorogenic acid was found to be absorbed from the small intestine. Only traces of chlorogenic acid were found in urine, suggesting an effective metabolism prior to urinary excretion. Maximum concentrations of rosmarinic acid in plasma were reached rapidly after oral administration: within 10 min in rats (*21*) and 30 min in humans (*22*), which indicates that at least some rosmarinic must be absorbed from the beginning of the digestive tract. This suggests that rosmarinic acid might also be a parent compound for metabolites excreted rather rapidly in urine.

In studies where rosmarinic acid or *Perilla frutescens* extract containing rosmarinic acid were orally administered to rats or humans, the absorption of rosmarinic acid was reported to be low (*17*−*22*). Because rosmarinic acid was orally administered to rats, the following metabolites were found in urine: rosmarinic acid, caffeic acid, *m*-hydroxyphenylpropionic acid, and sulfate conjugates of caffeic, ferulic, and *m*-coumaric acids (*17*). No metabolites attributed to rosmarinic acid could be found in the bile, suggesting that rosmarinic acid orally administered to rats was excreted in the urine rather than in the bile (*17*, *19*). The proportion of rosmarinic acid in rat faeces was 14% of the administered dose (*19*). After oral administration of *P. frutescens* extract containing rosmarinic acid to rats, the following urinary metabolites were reported: sulfate conjugates of *p*-coumaric, *m*-coumaric, ferulic, and caffeic acids, *m*-hydroxyphenylpropionic acid, apigenin, and luteolin (*18*). In the same study, *P. frutescens* extract administered to humans resulted in urinary excretion of only two metabolites: glucuronide derivatives of apigenin and trimethoxycinnamoyl acid. On the basis of this finding, a metabolic difference between rats and humans was suggested. However, in another human study (*22*), the metabolites found were close to those reported in rats: rosmarinic acid, methylated rosmarinic acid, and conjugated forms of caffeic, ferulic, and *m*-coumaric acids. This does not support the theory on the metabolic difference between rats and humans. On the basis of recent *in vitro* and rat studies (*16*, *21*, *28*), the majority of rosmarinic acid was suggested to be metabolized and degraded into *m*-coumaric acid and hydroxylated phenylpropionic acids by gut microflora, which then are efficiently absorbed. The metabolism of rosmarinic acid in humans (*22*) was suggested to involve microbial esterase in the digestive tract, hydrolyzing ester linkages in rosmarinic acid with minimal *p*-dehydroxylation of caffeic acid occurring in the lower portion of the gastrointestinal tract. The resulting caffeic and *m*-coumaric acids were suggested to be subsequently absorbed, conjugated, and methylated in tissues such as the digestive tract and liver, resulting in a variety of metabolites, for example, sulfated and glucuronide conjugates of caffeic, ferulic, and *m*-coumaric acids, which are then rapidly excreted in urine. The reported main metabolites of rosmarinic acid in previous studies (*17*, *18*, *20*, *22*) were minor metabolites in our study or did not exist.

The excreted caffeic acid probably derived from different chlorogenic acid forms and/or rosmarinic acid present in the extract. The low excretion (8 μmol) suggests that it was not a major metabolite of rosmarinic acid. Caffeic acid might have been further methylated to ferulic acid of which 25% (11 μmol) was derived from the metabolism of other phenolic compounds. Urinary ferulic acid may be derived from dietary sources or

from the metabolism of caffeic acid derived from caffeic acid esters (*36*). Chlorogenic acid was not detected in the urine samples. The only coumaric acid derivative detected in urine was *p*-coumaric acid, whereas *m*-coumaric acid, which has been previously reported in other studies, was not detected. In our earlier studies, *m*-coumaric acid has been seldom observed in human urine. The metabolism of *p*-coumaric acid was almost complete: after subtraction of the baseline excretion, only 3 μmol of the administered 23 μmol was rapidly excreted as such. Metabolism of *p*-coumaric acid to *p*-hydroxybenzoic acid would be possible, as well as hydroxylation followed by methylation. Syringic acid was not present in the extract, and the baseline excretion was low, indicating that the rapidly excreted urinary syringic acid was a metabolite of some phenolic compounds present in the extract. The potential parent molecule for syringic acid was gallic acid, which was not detected in the urine samples but was present in the oregano extract.

Protocatechuic acid was present in the extract, and half of the administered amount was rapidly excreted as such. Possible metabolites of protocatechuic acid were vanillic acid and *p*-hydroxybenzoic acid. The excretion of 3,4-dihydroxyphenylacetic acid increased during the first 4 h after the supplementation, which might indicate that it was produced from the lactic acid residue of rosmarinic acid. Also caffeic acid moieties could form 3,4-dihydroxyphenylacetic acid and *m*-hydroxyphenylacetic acid by metabolism occurring in the colon (*33*). However, neither of these compounds was found in human urine after the ingestion of coffee (*33*) or after oral administration of caffeic acid in rats (*36*), whereas several studies suggest these compounds to be specific metabolites for colonic metabolism of flavonols (*36*, *38*−*40*). *m*-Hydroxyphenylacetic acid was mainly excreted during the second day of the follow-up, suggesting that it was derived from colonic metabolism. Some unknown flavonoids possibly present in the extract might have partly contributed to the production of *m*-hydroxyphenylacetic acid. Potential parent compounds of *m*-hydroxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid in our study include both phenolic acids and flavonoids present in the extract.

The main compound excreted within 24 h after the supplementation was *p*-hydroxybenzoic acid (**Figure 4**), the mean excretion of which at the baseline was 21 μmol/day, whereas the excretion during the first 24 h of the follow-up was 225 μmol. During the second day of the follow-up, the excretion of *p*-hydroxybenzoic acid had returned to a level of 30 μmol/day. The excretory peak of *p*-hydroxybenzoic acid and also some minor metabolites appearing rapidly (2−6 h) after ingestion of the supplement suggest that these metabolites were not derived from phenolic metabolism in the colon but that the parent compound(s) had to be absorbed in the upper part of the gastrointestinal tract, quickly metabolized further, and excreted in urine. As mentioned earlier (*37*), *p*-hydroxylated metabolites of polyphenols have not often been described because of a preferential *p*-dehydroxylation of compounds by the gut microflora, an activity that has been located in the lower portion of the gastrointestinal tract (*41*). This is probably why *p*-hydroxybenzoic acid has also often been excluded from the list of monitored phenolic analytes. Earlier studies on rosmarinic acid or herbal extracts containing rosmarinic acid have not reported this compounds to be found in urine. No increase in the excretion of *p*-hydroxybenzoic acid in humans was reported after orally administered chlorogenic acid, quercetin-3-rutinoside or black tea solids (*42*), or chocolate (*43*). After supplementation with chlorogenic or caffeic acid in rats, *p*-hydroxybenzoic acid was not detected in urine, suggesting that it is not a metabolite of caffeic or quinic acid (*37*). However, because rosmarinic acid was the main phenolic component in the supplemented extract and *p*-hydroxybenzoic acid was the main metabolite found in urine, we suggest that *p*-hydroxybenzoic acid might derive from the metabolism of rosmarinic acid in humans. Small amounts of benzoic acid derivatives, including *p*-hydroxybenzoic acid, have been found in rat urine after supplementation with protocatechuic acid (*44*). Also supplementation with catechin and wine extract powder resulted in an increase in the excretion of *p*-hydroxybenzoic acid in rats (*45*). The metabolism of *p*-coumaric acid from aromatic amino acids tryptophan, tyrosine, and phenylalanine has been described to result in the formation of 4-hydroxybenzoic, 4-hydroxyhippuric, and hippuric acids (*33*, *46*).

The mixtures of phenolic compounds in their natural sources are complex. Such supplements are more of a practical and clinical relevance as compared to supplements consisting of pure compounds. However, studying the absorption and metabolism of these types of sources of phenolic compounds is challenging, because a number of parent compounds, normally only partly identified, are converted to a variety of metabolites. In animal studies on rosmarinic acid, the administered amounts have been massive as compared to amounts ingested in humans (*17*−*22*), and also differences in metabolism between species have been discussed (*18*). However, different doses of phenolic supplements used (*27*) and variation in the monitored metabolites and methods of analysis complicate the comparison between studies and make the interpretation of results challenging. Our results show that phenolic acids from *O. onites* extract were absorbed but were also quickly metabolized in the body, and thus, elucidation of the antioxidant action of these metabolites must be emphasized in further work. The fact that most of the metabolites were rapidly excreted suggests that potential antioxidant effects in the human body would be of short-term nature. The bioactivity of the metabolites present in the body and finally in urine or feces differ from that of their parent compounds. Even though the antioxidant capacity of phenolic compounds is decreased during the metabolism, metabolites often still contain hydroxyl groups capable of antioxidant action. Most of the compounds originating from the metabolism of the oregano extract in humans were potent antioxidants, although less potent than parent compounds. They were also for example capable of *in vitro* radical scavenging action as well as inhibition of low-density lipoprotein oxidation *in vitro*. Compounds that are poorly absorbed are found in higher concentrations in the content of the end of the gastrointestinal tract and may thus exert some beneficial effects on the tract itself, although not affecting health via the systemic blood circulation. The same concerns phenolic metabolites that are formed by microbial metabolism in the gut. Concentrations of a variety of phenolic acids, for example, have been found to be very high in human fecal water (*47*), and the antioxidant or other protective effects of phenolic compounds could contribute to the observed protection against cancers in the gastrointestinal tract (*48*). To evaluate the health effects of herbal extracts or constituents thereof, different metabolites should be studied as such and also in conjugated forms, preliminarily *in vitro* and then in human studies. Subject of interest in the future will also be to study whether the phenolic compounds absorbed from the extract or metabolites thereof really contribute to the antioxidant status of the body at the concentrations present in humans after supplementation.

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