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Benefits of the ipowder® extraction process applied to *Melissa officinalis* L.: improvement of antioxidant activity and *in vitro* gastro-intestinal release profile of rosmarinic acid

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The objective of this study was to evaluate the benefits of a new extraction process, the ipowder® technology, applied to *Melissa officinalis* L. Compared to *M. officinalis* ground dry leaves, the ipowder® had a similar phytochemical fingerprint but contained twice the concentration of rosmarinic acid (by HPTLC and HPLC) and had a two-fold greater antioxidant activity (DPPH* method). *In vitro* digestion experiments (TIM-1 model) showed better availability of rosmarinic acid for intestinal absorption with the ipowder® than with ground dry leaves, manifested by a three-fold reduction in the quantity of ingested product needed for delivery of the same amount of rosmarinic acid into the upper gastro-intestinal tract. This study shows that the ipowder® technology preserves all the original plant compounds intact while making some active ingredients more accessible and available to exert their effects. To obtain a given effect, the amount of ipowder® extract to ingest will therefore be lower; a reduction in the daily dosage will be more convenient for the patient and will improve patient compliance with supplementation.

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1. Introduction

Herbs have been used as therapeutic agents all over the world for thousands of years. The World Health Organization estimated that 21 000 plant species could potentially be used for medicinal purposes and that over 80% of people worldwide rely on herbal medicines for their everyday health care needs.^{1,2} It has also been estimated that herbal products account for 25% of total medicine consumption in developed countries and up to 80% in developing countries such as India or China.

Medicinal plants are used either directly as dry plant powder or as liquid or dry formulations of their active ingredients extracted by various methods. The amount of dry plant powder to be ingested in order to achieve a physiological effect is generally relatively high, especially since the bioavailability of the active compounds is usually low. Moreover, in the case of an extract, its quality depends greatly on the extraction

method. According to the method used, different active compounds will be extracted, at varying concentrations.

Loss and degradation of certain plant ingredients often occur during the preparation of extracts and as a result, their phytochemical composition differs from that of the totum, defined as the entire set of active compounds contained in the part of the plant used for extraction. A fundamental principle of phytotherapy is that of synergy, according to which the activity of the totum is more than the sum of the effects of the individual active ingredients taken separately. It is therefore essential to design new processes for the preparation of plant extracts that preserve intact in the finished product all the active substances contained in the original medicinal plant while increasing their availability. The ipowder® technology was developed for this purpose.

The ipowder® process consists of three essential steps, namely contact between the plant material and a solvent, at least one step of extraction of the active compounds, and finally spray-drying of the resulting extract on to the same plant material as that used to produce the extract.³ This plant material is then crushed to form the ipowder®. The spray-drying procedure yields a final product enriched in active substances and also guarantees the presence of all the active compounds contained in the original plant material used. In contrast, the composition of dry plant extracts prepared by conven-

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tional methods generally differs from the totum because these extracts are dried on an inert support (such as maltodextrin, starch *etc.*) or without using any support.

The objective of this study was to show that the ipowder® technology: (1) concentrates certain active ingredients while preserving all the active compounds contained in the original plant material without altering these; (2) enhances their biological effects; and (3) increases the availability of the active compounds for intestinal absorption during digestion. For this purpose, we applied the process to leaves of *Melissa officinalis* L., also known as lemon balm, a medicinal plant that has long been used in traditional medicine. *M. officinalis* has sedative, spasmolytic, and hypotensive properties, as well as fever-reducing, thyroid-related, antiviral and antioxidant activities, and has been shown to be of benefit against asthma, heart failure, ulcers and wounds.^{4–6} *M. officinalis* is also used for the symptomatic treatment of mild gastro-intestinal complaints, including bloating and flatulence,⁷ and for the symptomatic treatment of digestive disorders such as minor spasms.⁸ Extracts of *M. officinalis* leaves contain substances belonging to various chemical classes, such as triterpenes, flavonoids and polyphenolic compounds.⁹ The major compound present in leaf extracts, identified as rosmarinic acid (RA), is commonly used as a marker.⁹

To show the benefits of the ipowder® technology, we determined the phytochemical fingerprint of the *M. officinalis* ipowder® (hereafter referred to as ipowder®), RA concentration, antioxidant activity and release of RA during *in vitro* digestion (using the TIM-1 model that mimics the human digestion process), and compared the characteristics and properties of this ipowder® to those of *M. officinalis* ground dry leaves.

2. Materials and methods

2.1. Plant collection, identification and extraction

The leaves of *M. officinalis* were collected in Aubiat (France) in June 2015 and identified by Gilles Thébault from the herbarium of the Museum d'Histoire Naturelle Henri-Lecoq (Clermont-Ferrand, France) in which a voucher specimen was deposited (CLF106452). This herbarium is registered with the International Association for Plant Taxonomy, the head office of which is located in the New York botanical garden.

The extract of *M. officinalis* tested (Lemon balm ipowder®, PiLeJe Industrie, France³) is obtained by extraction of 1 kg of *M. officinalis* cut dry leaves in 10 L of water at 85 °C for 30 minutes. After filtration, the resulting extract is concentrated under vacuum (native extract ratio [NER]: 5 to 7 : 1), then fixed and dried on 0.5 kg of *M. officinalis* cut dry leaves (impregnation support) under reduced pressure (drug extract ratio [DER]: 2 to 4 : 1). The enriched plant material is finally crushed to form the ipowder® and filled into capsules.

2.2. Chromatographic fingerprinting

All chemicals were purchased from Sigma Aldrich (USA).

2.2.1. High-performance thin-layer chromatography (HPTLC). Analyses were performed using 0.20 mm silicagel 60 (20 × 10 cm) glass HPTLC analytical plates (Merck, Darmstadt, Germany). Material included a CAMAG HPTLC system (Muttentz, Switzerland) equipped with an Automatic TLC Sampler (ATS 4), an Automatic Developing Chamber (ADC2) with humidity control, a TLC Visualizer, VisionCATS software and for derivatization, a Chromatogram Immersion Device III and TLC Plate Heater III.

Samples were obtained from the analytical extraction of 1 g of *M. officinalis* ground dry leaves or ipowder® in 100 mL of different solvents (water, ethanol/water: 50/50 v/v [50% ethanol] or methanol). After a 15 min sonication at room temperature, the samples were filtered. The RA standard solution was prepared from 10 mg of RA in 200 mL of 50% ethanol and sonicated for 5 min.

Test solutions (10 µL) and RA standard solution (8 µL) were applied on 8 mm bands, 8 mm from the lower edge of the plate. The mobile phase was a mixture of ethyl acetate, water, acetic acid and formic acid (100/27/11/11). Plates were developed over a distance of 70 mm from the lower edge using a twin trough glass chamber saturated for 20 min with the mobile phase under controlled humidity (RH: 33%). After development, plates were dried under a stream of cool air for 10 min. The plates were heated at 100 °C for 3 min then immersed in Natural Product (NP) reagent (1 g of 2-aminoethyl diphenylborinate in 200 mL of ethyl acetate) at a speed of 5 cm s⁻¹ with an immersion time of 0 s, then dried under a stream of cool air. The plates were immersed in polyethylene glycol (PEG) reagent (10 g of PEG 400 in 200 mL of dichloromethane) at the same speed and immersion time then dried. Digital images were taken at 366 nm.

The same HPTLC conditions were used to detect antioxidant activity. After development, the plate was immersed in a 0.5 mM methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution with a speed of 3 cm s⁻¹ and an immersion time of 5 s. The plate was dried at room temperature in the dark for 90 s and then heated for 30 s at 60 °C. The chromatogram was analysed under white light (reflectance mode).

2.2.2. LC/MS system and conditions. For LC/MS analyses, the ipowder® (1 g) was extracted with 10 mL of 50% ethanol under sonication for 10 min at room temperature, protected from light. After centrifugation for 5 min at 3000g, the solution was filtered through 0.45 µm syringe polytetrafluoroethylene (PTFE) filters and then diluted 10-fold.

Chromatographic analyses using ultra-high-performance liquid chromatography (UHPLC) were performed on an Ultimate 3000 RSLC UHPLC system (Thermo Fisher Scientific Inc., MA, USA) coupled to a quaternary rapid separation pump (ultimate autosampler) and a rapid separation diode array detector. Compounds were separated on an Uptisphere Strategy C18 column (250 × 4.6 mm, 5 µm, Interchim, France), controlled at 30 °C. The mobile phase was a mixture of 0.1% (v/v) formic acid in water (phase A) and 0.1% (v/v) formic acid in acetonitrile (phase B). The gradient of phase A was 100% (0 min), 80% (10 min), 73% (35 min), 0% (40–50 min) and



100% (51–60 min). The flow rate was 0.8 mL min⁻¹, and the injection volume was 5 µL. The UHPLC system was connected to an Orbitrap (Thermo Fisher Scientific Inc., MA, USA) mass spectrometer, operated in the negative electrospray ionization mode. Source operating conditions were: 3 kV spray voltage; 320 °C heated capillary temperature; 400 °C auxiliary gas temperature; sheath, sweep and auxiliary gas (nitrogen) flow rate 50, 10 and 2 arbitrary units, respectively; and collision cell voltage between 10 and 50 eV. Full scan data were obtained at a resolution of 70 000 whereas MS² data were obtained at a resolution of 17 500. Data were processed using Xcalibur software (Thermo Fisher Scientific Inc., MA, USA).

2.3. Quantification of RA

M. officinalis ground dry leaves or ipowder® (200 mg of each) were extracted with 200 mL of 50% ethanol and the extracts were sonicated for 1 h at room temperature, protected from light. The extracts were then filtered and diluted with the same solvent to 200 mL. The resulting solutions were then filtered through 0.45 µm syringe PTFE filters.

2.3.1. HPTLC. The standard solution of RA was prepared from 10 mg of RA in 180 mL of 50% ethanol, sonicated for 5 min and completed with the same solvent to 200 mL. The standard range, comprising volumes of 2, 4, 6, 8 and 10 µL of the standard RA solution and the test solutions (15 µL) were applied as 8 mm bands, 8 mm from the lower edge of the plate. The mobile phase was a mixture of toluene, ethyl formate and formic acid (6/4/1). Plates were developed over a distance of 80 mm from the lower edge using a twin trough glass chamber saturated for 20 min with the mobile phase under controlled humidity (RH: 33%). After development, plates were dried under a stream of cool air for 10 min in the developing chamber. Digital images were captured at 366 nm. UV/Vis spectra (200–700 nm) of RA were recorded to determine their maximum absorbance wavelengths using a TLC Scanner 4. Absorbance was measured at 330 nm.¹⁰ All assays to determine the quantity of RA in the ground dry leaves and in ipowder® were carried out in triplicate on three different plates.

2.3.2. HPLC. Chromatographic analyses were performed using a Symmetry C18 Cartridge, 100 Å, 5 µm, 4.6 mm × 250 mm (Waters, Ireland) with a Waters HPLC system comprising a 600 system controller, a 600 pump, an In-Line Degasser, a 717 plus Autosampler and a 996 Photodiode Array Detector equipped with a DAD detector (190 to 400 nm). Analyses were performed at 20 °C. The mobile phase was a 2% solution of acetic acid in water (A) and acetonitrile (B) for RA quantification. The gradient comprised a 0–20 min linear gradient from 90–10% (A–B) to 50–50%, a 20–21 min gradient to 10–90% (A–B) and then a 21–40 min isocratic gradient (90% B). The flow rate was set at 1 mL min⁻¹ and absorbance was monitored at 352 nm.

The RA standard solution was prepared from 22.4 mg of RA in 50 mL of a mixture of 50% ethanol and 50% water, acidified by the addition of 2% glacial acetic acid (ethanol/acidified water). The mixture was then sonicated for 5 min. The RA calibration curve was obtained by analysing samples containing

25 to 400 mg L⁻¹ of RA in the same solvent. The solutions were filtered through a 0.45 µm PTFE membrane filter prior to injection. All assays to determine the quantity of RA in the ground dry leaves and ipowder® were carried out in triplicate.

2.4. DPPH* method

The DPPH* free radical scavenging method allows evaluation of the antioxidant potential of a compound or an extract. Analyses were performed using a microplate reader (Tecan M Nano – Infinite 200 pro, Lyon, France) at 515 nm and flat, transparent 96-well plates with lids (Greiner, Kremsmunster, Austria).

For sample preparation, 0.5 g of *M. officinalis* ground dry leaves or ipowder® were extracted with 45 mL of 50% ethanol, under sonication for 30 min at room temperature. The extracts were then filtered, the volume was completed to 50 mL and finally diluted 75 times with the same solvent. Standard solutions of Trolox (2.35 to 150 µM) were prepared in methanol.

Aliquots of the standard or sample solutions (50 µL) were introduced into the wells with 250 µL of DPPH* solution (79 µM in methanol). The plates were kept for 30 min in the dark at room temperature. The absorbance (Abs) was measured at 515 nm and converted into the percentage of inhibition of DPPH* radical using the following formula:

$$\text{Scavenging activity(\%)} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100.$$

A DPPH* solution (100 µL) in methanol (100 µL) was used as the control (Abs control). All assays were performed in triplicate and results were reported as the mean ± standard deviation (SD). DPPH* scavenging activities were expressed as mg of Trolox equivalents per g of samples using a linear regression curve.

2.5. In vitro gastro-intestinal digestion

The TNO gastro-Intestinal Model (TIM-1) is a multi-compartment, dynamic and computer-controlled system developed at TNO Nutrition and Food Research (Zeist, the Netherlands) to reproduce the digestive process occurring in the luminal part of the human upper digestive tract, as previously described.^{11–13} Briefly, the system consists of four successive compartments simulating the stomach, duodenum, jejunum and ileum. It enables control of the main mechanical, physical and biochemical parameters of digestion such as pH, temperature, peristaltic movements, volumes, transit times of the chyme, digestive secretions and passive absorption of water, salts and soluble small compounds through hollow fibres that continuously dialyse the jejunal and ileal contents. The TIM-1 system was set to simulate the digestion of a glass of water in a healthy human adult for a total duration of 4 h (Table 1).

In this model, we compared the amount of RA released from a specified quantity of ipowder® with that released from the total quantity of ground dry plant leaves required to produce this quantity of ipowder®. As described in section 2.1, the ipowder® is produced by spray-drying of an extract obtained from two parts of *M. officinalis* leaves on one part of *M. officinalis* leaves meaning that one part of ipowder® is



Table 1 Digestive conditions applied for *in vitro* digestion in the TIM-1 system. The TIM-1 was programmed to reproduce the digestion of a glass of water in a healthy adult

Compartment	Volume (mL) at initial time	pH/time (min)	Secretions	<i>T</i> (min); β coefficient ^a
Stomach	10 gastric residue 200 water	1.8/0; 6.0/1; 3.2/10; 2.4/20; 1.8/40; 1.6/60; 1.5/240	Pepsin: 130 IU min ⁻¹ Lipase: 7.5 IU min ⁻¹ 0.5 M HCl when necessary	20; 1
Duodenum	50	Maintained at 6.4	Bile extract (porcine): 40 mg min ⁻¹ on 0–25 min, then 20 mg min ⁻¹ Pancreatin 4 USP: 22.3 mg min ⁻¹ 0.5 M NaHCO ₃ when necessary	
Jejunum	130	Maintained at 6.9	0.5 M NaHCO ₃ when necessary	
Jejunal dialysis	10 mL min ⁻¹		5 mM <i>K</i> -phosphate buffer pH 6.9; NaCl 5 g L ⁻¹ ; CaCl ₂ , 2H ₂ O 0.2 g L ⁻¹	
Ileum	130	Maintained at 7.2	0.5 M NaHCO ₃ when necessary	150; 2.4
Ileal dialysis	10 mL min ⁻¹		5 mM <i>K</i> -phosphate buffer pH 7.2; NaCl 5 g L ⁻¹ ; CaCl ₂ , 2H ₂ O 0.2 g L ⁻¹	

^a Mathematical modelling of gastric and ileal deliveries with power exponential equation was used for the computer control of chyme transit: $f = 1 - 2^{-(t/T)^{1/\beta}}$ where f represents the fraction of the meal delivered, t the time of delivery, T the half-time of delivery and β a coefficient describing the shape of the curve.

obtained from three parts of raw plant material. HydroxyPropylMethylCellulose (HPMC) capsules size 00 were filled with 300 mg of *M. officinalis* ground dry leaves or ipowder®. One capsule of ipowder® or three capsules of leaves were digested with 200 mL of mineral water (Volvic®, Volvic, France) in the TIM-1 system, in triplicate. During digestion, cumulated dialysis fluids (jejunal and ileal dialysates) and cumulated ileal effluents were regularly collected. Volumes were measured and samples were stored at -20 °C for downstream analysis of RA concentration. After 4 h, the final gastrointestinal contents of the four compartments of the TIM-1 system were pooled and a sample was also frozen for analysis.

3. Results and discussion

3.1. Fingerprint

M. officinalis ground dry leaves and ipowder® were extracted with three different solvents (methanol, water and 50% ethanol) in order to determine the best analytical extraction conditions for studying their fingerprints by HPTLC. The use of 50% ethanol allowed optimal extraction of phenolic compounds (Fig. 1a, lanes 3 and 6). The comparison between ground dry leaves and ipowder® profiles showed that the ipowder® fingerprint was equivalent to that of the ground dry leaves but that the concentration of secondary metabolites was higher in the ipowder®. The ipowder® technology therefore preserves the fingerprint of ground dry leaves and concentrates secondary metabolites.

The immersion of a second HPTLC plate in DPPH* (Fig. 1b) showed that both ground dry leaves and ipowder® have antioxidant activities with RA as the major marker of this activity (upper white spots on lanes 1, 3, 5, 6, 7). HPTLC analysis showed the presence of various polyphenol derivatives including RA. As previously described, RA was the major compound detected in the extracts of *M. officinalis* analysed.^{9,14,15}

The phytochemical profile of ipowder® was determined by LC/MS analyses in the negative ionization mode using the optimal extraction solvent (*i.e.* 50% ethanol; Fig. 1c and Table 2). The LC/MS spectrum showed the presence of RA (Fig. 1c, signal 11; *rt*: 26.38 min *M-H*: 359.0773). Other compounds identified included RA derivatives such as danshensu (Fig. 1c, signal 5, *M-H*: 197.0450), and 3'-*O*-(8''-*Z*-caffeoyl) rosmarinic acid (Fig. 1c, signal 12, *M-H*: 537.1042) and flavones such as luteolin 3'-*O*- β -D-glucuronide (Fig. 1c, signal 9, *M-H*: 461.0732).

3.2. Quantification of RA by HPLC and HPTLC

The quantity of RA in *M. officinalis* ground dry leaves and ipowder® was measured by HPLC and HPTLC (Table 3). HPTLC allows rapid and efficient quantification of markers. RA was quantified using three different plates and five standard concentrations.

Both HPTLC and HPLC analyses showed that the ipowder® contained a higher concentration of RA than the ground dry leaves. The concentration of RA detected in the ipowder® was 2.62 ± 0.05 mg of RA per 100 mg of dried raw material by HPTLC and 2.76 ± 0.05 mg by HPLC. These concentrations were almost twofold higher than in the ground dry leaves (1.45 ± 0.10 mg of RA per 100 mg of dried raw material by HPTLC and 1.34 ± 0.07 mg by HPLC). It is worth noting that the two quantification methods resulted in the detection of similar concentrations of RA. The ratio of 1.8 between the RA concentrations in the ipowder® and in the ground dry leaves detected with HPTLC was equivalent to the ratio of 2.1 obtained with HPLC.

3.3. Antioxidant activity

Antioxidant activity was evaluated using the DPPH* test, based on the ability of metabolites to donate a hydrogen atom or electron to the stable radical DPPH*. The assay evaluates the ability of *M. officinalis* extracts to scavenge free radicals in solution. We also assessed the Trolox equivalent antioxidant capacity of the extracts.



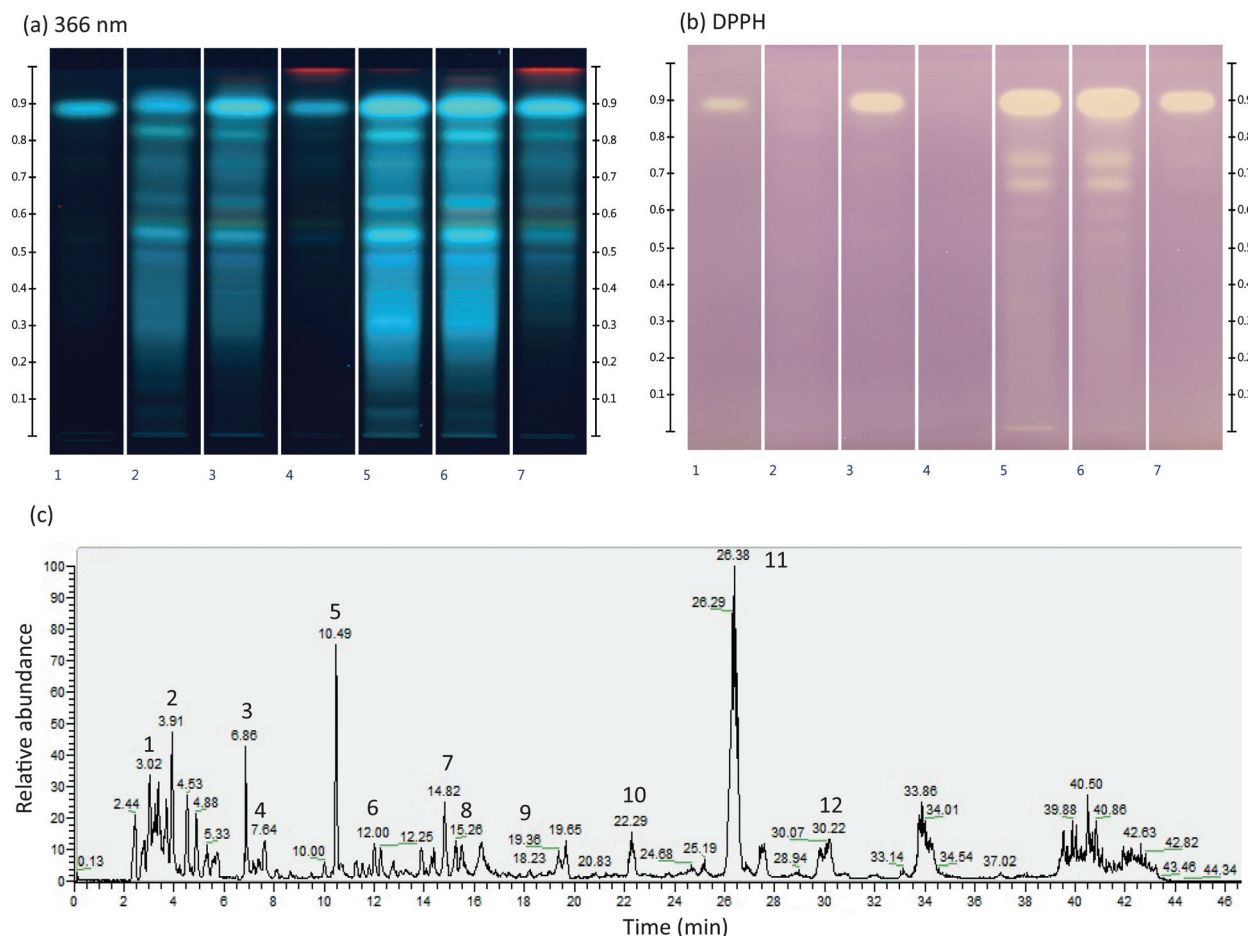


Fig. 1 (a) and (b) Chromatographic fingerprint of analytical extracts of *M. officinalis* ground dry leaves (lanes 2–4) and ipowder® (lanes 5–7) with three extraction solvents (water: lanes 2 and 5; 50% ethanol: lanes 3 and 6; methanol: lanes 4 and 7) at 366 nm (a) and with DPPH* method (b). Lane 1: rosmarinic acid standard. (c). LC/MS spectrum of ipowder® (see Table 2 for signal identification).

Table 2 Identification of the major compounds of *M. officinalis* ipowder® with UHPLC-MS and MS² in the negative ionization mode

Peak	Retention time (min)	Molecular ion [M-H] ⁻ (m/z)	Formula	MS ² (m/z)	Compounds	Ref.
1	3.68	149.0076	C ₄ H ₆ O ₆	149/87/72/59/103	Tartaric acid	Standard
2	3.91	191.0549	C ₇ H ₁₂ O ₆	191/85/127	Quinic acid	Standard
3	6.86	191.0192	C ₆ H ₈ O ₇	111/87/85/191	Citric acid	Standard
4	7.64	117.0182	C ₄ H ₆ O ₄	73/117/99	Succinic acid	16
5	10.49	197.0450	C ₉ H ₁₀ O ₅	72/135/123/179	Danshensu	17
6	12.00	311.0411	C ₁₃ H ₁₂ O ₉	149/179/135/87	Caftaric acid	18
7	14.82	179.0341	C ₉ H ₈ O ₄	135	Caffeic acid	Standard
8	16.04	537.1046	C ₂₇ H ₂₂ O ₁₂	295/179/135/121/493	Lithospermic acid A	19
9	19.47	461.0732	C ₂₁ H ₁₈ O ₁₂	285	Luteolin 3'-O-β-D-glucuronide	20
10	22.29	719.1618	C ₃₆ H ₃₂ O ₁₆	161/359/197/179/135/341/133	Sagerinic acid	21
11	26.38	359.0773	C ₁₈ H ₁₆ O ₈	161/197/179/135	Rosmarinic acid	Standard
12	30.22	537.1042	C ₂₇ H ₂₂ O ₁₂	161/135/359/179/197	3'-O-(8''-Z-Caffeoyl) rosmarinic acid	22

The ipowder® exhibited a scavenging activity that was twofold greater than that of the ground dry leaves (Table 3). A similar result was obtained for the Trolox equivalent.

Altogether these results showed that the antioxidant activity of ground dry leaves and ipowder® was correlated with the concentration of RA: with a RA concentration twice as high as

in ground dry leaves, the ipowder® had a scavenging activity two-fold greater.

3.4. Release of RA during *in vitro* gastro-intestinal digestion

The ability of *M. officinalis* ipowder® to release RA into the gastro-intestinal tract (GIT) was compared to that of



Table 3 Rosmarinic acid (RA) quantity, scavenging activity percentage and Trolox equivalent of the *M. officinalis* ground dry leaves and ipowder® (means \pm SD, $n = 3$)

	<i>M. officinalis</i> leaves	ipowder®
mg of RA per 100 mg of dried raw material		
Measured by HPTLC	1.45 \pm 0.10	2.62 \pm 0.05
Measured by HPLC	1.34 \pm 0.07	2.76 \pm 0.05
% scavenging activity	21.6 \pm 0.6	44.9 \pm 1.8
Trolox equivalent (mg g ⁻¹ sample)	291 \pm 10	618 \pm 23

M. officinalis ground dry leaves (plant equivalent) using the TIM-1 model, a dynamic computer-controlled system simulating the main spatio-temporal digestive parameters occurring into the upper GIT of humans. The TIM-1 system is a relevant *in vitro* model previously used to study the release of chemical compounds from food matrices and drugs;^{23,24} it was used for the first time to investigate a plant extract in this study.

RA release and dialysis from the two preparations were similar as no difference in the kinetic profiles and total quantities dialysed were observed (Fig. 2 and Table 4). This observation is not due to system saturation since other studies have shown that higher amounts of solubilized substances can be dialysed in the TIM-1 model.²⁴ Moreover, we observed in a preliminary digestion on a dry extract of *M. officinalis* leaves (60–80% native extract, 30% ethanol) that about 12 mg of RA for an ingested quantity of 13.3 mg was solubilized and dialysed in the TIM-1 system (data not shown). One capsule of ipowder® (300 mg) therefore released the same amount of RA as three capsules containing *M. officinalis* ground dry leaves (900 mg in total). This result is of great interest as it shows that the novel ipowder® extraction process will enable reduction of the recommended daily intake of this herbal product.

In both cases, RA was mainly delivered in the proximal part of the GIT since 83 and 84% of RA from the ground dry leaves and the ipowder®, respectively, were recovered in the jejunal

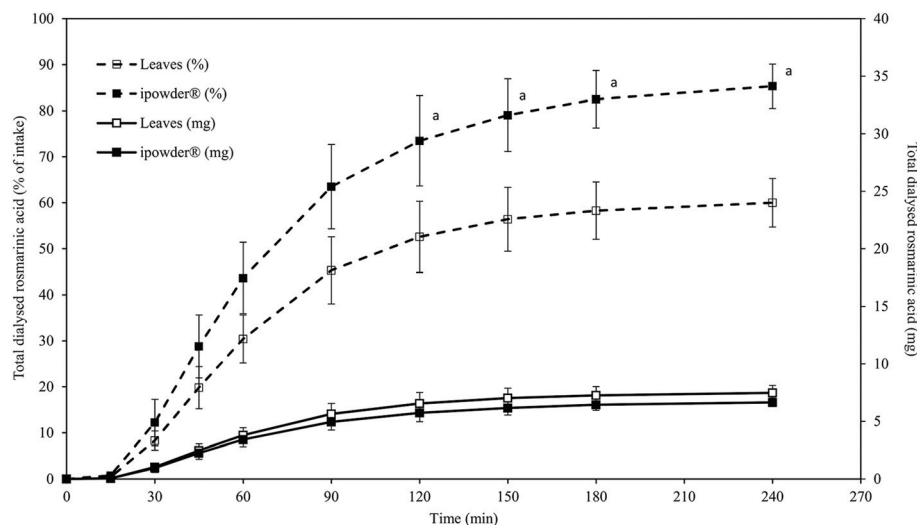


Fig. 2 Cumulative amount of RA released from *M. officinalis* ground dry leaves and ipowder®, and dialysed during *in vitro* digestion using the TIM-1 system. The quantities of RA are expressed in mg of the total compound (continuous line) and as percentages of initial intake (dotted line). Values are means of three independent experiments with standard deviations. a: Statistically significant difference between ipowder® and ground dry leaves at the same time point (tested by a student *t*-test, with a probability level of $P < 0.05$ considered to be statistically different).

Table 4 Mass balance of released RA from *M. officinalis* ground dry leaves and ipowder® after 4 h of digestion in the TIM-1 model. Values are means of three independent experiments with standard deviations

	<i>M. officinalis</i> ground dry leaves (3 capsules)		ipowder® (1 capsule)	
	Total RA \pm SD (mg)	% of intake \pm SD	Total RA \pm SD (mg)	% of intake \pm SD
Intake	12.44 \pm 0.02	100	7.79 \pm 0.10 ^a	100
Cumulated dialysates	7.5 \pm 0.7	60.0 \pm 5.3	6.6 \pm 0.4	85.3 \pm 4.8 ^a
Cumulated ileal effluents	0.07 \pm 0.02	0.6 \pm 0.2	0.18 \pm 0.06 ^a	2.3 \pm 0.7 ^a
Final GIT content	0.27 \pm 0.04	2.2 \pm 0.3	0.25 \pm 0.24	3.2 \pm 3.1
Total release	7.8 \pm 0.6	62.7 \pm 5.1	7.1 \pm 0.5	90.8 \pm 6.5 ^a

^a Statistically significant difference between ipowder® and ground dry leaves (student *t*-test, with a probability level of $P < 0.05$ considered to be statistically different).



dialysates (data not shown). Only a small quantity of free RA was recovered in the cumulative ileal effluents during the 4 h of digestion, and at the end of digestion in the residual contents of the TIM-1 compartments, suggesting that RA was rapidly released from both formulations during digestion (Table 4).

However, compared to the total amount of RA contained in each product ingested, the percentage of recovery of the free biomarker was significantly higher for the ipowder® than for the ground dry leaves ($90.8 \pm 6.5\%$ for ipowder® vs. $62.7 \pm 5.1\%$ for the leaves). RA therefore seems to be more easily released from the ipowder® than from ground dry leaves during digestion and consequently more available for intestinal absorption. This is most likely due to the pre-extraction of RA (and other active compounds) in the case of the ipowder®. It should be noted that other compounds, in particular caffeic acid and danshensu with a chemical structure similar to that of RA, were also detected in the dialysates (data not shown).

4. Conclusion

The results of this study, focusing on *M. officinalis* ipowder®, demonstrate that the novel extraction technology based on a plant extract that is concentrated then fixed and dried on the source plant material gives a phytochemical fingerprint similar to that of the original plant material but with a higher concentration of RA, one of the main biologically active compounds of *M. officinalis*. We identified various chemical compounds (both phenolic and flavonoid) and quantified RA using two different methods: HPTLC and HPLC. These two methods gave comparable results, showing that the concentration of RA was two-fold higher in the ipowder® than in the ground dry leaves. Using the DPPH* method, we also demonstrated that the antioxidant properties of *M. officinalis* were improved in the ipowder® form due to the higher RA concentration. The absorption of RA during *in vitro* digestion, and possibly that of other compounds, was also improved with the ipowder® due to the higher percentage of release of the biologically active substance from this formulation. We have chosen to carry out our experiments under the simplest conditions, *i.e.* with water, because it is a usual way for patients to ingest food supplements. Nevertheless, it would be interesting in a further work to study the impact of a complex food matrix on the release and availability for absorption of rosmarinic acid.

Altogether, this study shows that the ipowder® technology preserves all the original plant compounds intact (nonselective extraction) while making some active compounds more accessible and available to exert their effects. The quantity of ingested product required to obtain the same amount of RA available for absorption was reduced by a factor of three. To obtain a given effect, the amount of *M. officinalis* ipowder® extract to be ingested will therefore be lower than in the case of ground dry leaves. The consequent reduction in daily dosage will result in greater convenience for the patient and will improve patient compliance with supplementation.

Conflicts of interest

Valérie Bardot, Anaïs Escalon, César Cotte, Michel Dubourdeaux are employees of PiLeJe Industrie. Isabelle Ripoché, Pierre Chalard, Lucile Berthomier, and Martin Leremboure performed the chromatographic analyses and Sylvain Denis, Monique Alric, Sandrine Chalancon performed the experiments using the TIM-1 system for PiLeJe.

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