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Effect of Phospholipid-Based Formulations of *Boswellia serrata* Extract on the Solubility, Permeability, and Absorption of the Individual Boswellic Acid Constituents Present

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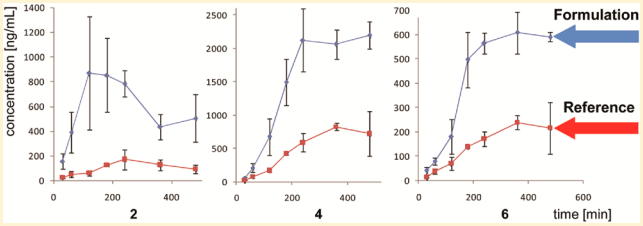
ABSTRACT: *Boswellia serrata* gum resin extracts are used widely for the treatment of inflammatory diseases. However, very low concentrations in the plasma and brain were observed for the boswellic acids (**1**−**6**, the active constituents of *B. serrata*). The present study investigated the effect of phospholipids alone and in combination with common co-surfactants (e.g., Tween 80, vitamin E-TPGS, pluronic f127) on the solubility of **1**−**6** in physiologically relevant media and on the permeability in the Caco-2 cell model. Because of the high lipophilicity of **1**−**6**, the permeability experiments were adapted to physiological conditions using modified fasted state simulated intestinal fluid as apical (donor) medium and 4% bovine serum albumin in the basolateral (receiver) compartment. A formulation composed of extract/phospholipid/pluronic f127 (1:1:1 w/w/w) increased the solubility of **1**−**6** up to 54 times compared with the nonformulated extract and exhibited the highest mass net flux in the permeability tests. The oral administration of this formulation to rats (240 mg/kg) resulted in 26 and 14 times higher plasma levels for 11-keto-β-boswellic acid (**1**) and acetyl-11-keto-β-boswellic acid (**2**), respectively. In the brain, five times higher levels for **2** compared to the nonformulated extract were determined 8 h after oral administration.



Boswellia serrata Roxb. (Burseraceae) gum resin extract is a popular remedy in Ayurvedic medicine. It is used widely by patients and recommended by physicians for the supplemental treatment of various inflammatory diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, and inflammatory bowel disease.[1,2] Several pilot clinical trials suggest promising beneficial therapeutic effects with no serious, long-term, or irreversible adverse effects.[3] Moreover, *B. serrata* significantly reduced peritumoral edema in preliminary clinical trials.[4−6] On the basis of these facts, *B. serrata* gum resin extract was accorded orphan drug status by the European Medicines Agency (EMA) in 2002. For some time 11-keto-β-boswellic acid (**1**) and acetyl-11-keto-β-boswellic acid (**2**) were believed to be responsible for the pharmacological activity of *B. serrata* as a result of inhibiting 5-lipoxygenase (5-LO). However, their plasma and brain concentrations were found to be very low following oral administration of even high *B. serrata* doses. In most cases, **2** was not detected at all.[7−12] Only β-boswellic acid (**3**) and acetyl-β-boswellic acid (**4**), which inhibit microsomal prostaglandin E_2 synthase (mPGES-1)[13] and cathepsin G (catG), achieved pharmacologically relevant concentrations in plasma.[7,14] On the basis of this background, the present study was aimed to develop a formulation that enhances the absorption of boswellic acids from *B. serrata* extract.

A prerequisite for high absorption is sufficient drug solubility in the intestinal fluid. This depends on the drug formulation and on the composition of the intestinal fluid, which is influenced by food intake. Hence a 3-fold increase in the bioavailability of lipophilic boswellic acids was reported for the concomitant administration of *B. serrata* extract with fatty meals.[11] The observed food effect may be attributed to the direct solubilizing effects of the food ingredients (e.g., triglycerides) and/or to the stimulation of endogenous biliary-derived bile salts and phospholipids.[14,15] In addition, the delayed gastric emptying following food intake prolongs the solubilization time.[16] Phospholipids, especially phosphatidylcholine, triacylglycerols, bile acids, and cholesterol, are mainly responsible for the solubilizing effects by the formation of micelles, which results in the diffusion of water-insoluble substances through the "unstirred water layer" covering the brush border membrane.[17] Phosphatidylcholine, endogenously delivered by bile (11−12 g/day) and diet (1−2 g/day), is a generally recognized as safe (GRAS) substance.[18] Due to its amphiphilic behavior, it represents the main constituent of liposomal formulations and of the so-called phytopharmaceutical−phospholipid complexes

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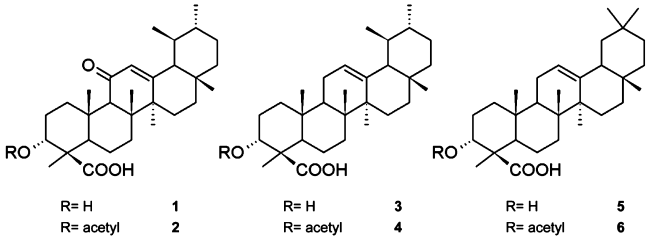
[e.g., Phytosome, a trademark registered by Indena (S.p.A.)].[19,20] Even if these systems are described as complexes, no isolated 1:1 drug−phospholipid complexes are formed from a chemical point of view, but the drug is located mainly in the lipophilic membrane area of the liposomal structure formed upon rehydration of the so-called phytopharmaceutical−phospholipid complexes in aqueous media.[21] Compared to liposomes, these systems are characterized by a high drug/lipid ratio.[22] It has been reported that "complexation" with phospholipids improves the bioavailability and efficacy of several herbal extracts, such as *Ginkgo biloba* and milk thistle, as well as substances such as curcumin.[23−25]

On the basis of these results, the effect of phospholipid complexation on the solubility, permeability, and absorption of **1**−**6** was tested. Moreover, the influence of additional cosurfactants such as Tween 80, vitamin E D-alpha tocopheryl polyethylene glycol succinate (vitamin E-TPGS), and pluronic f127 was investigated.[26] The solubility of the boswellic acid−phospholipid complexes was determined in physiologically relevant media [e.g., in FaSSIF (fasted state simulated intestinal fluid), FeSSIF (fed state simulated intestinal fluid), and SGF (simulated gastric fluid)]. In addition, the permeability was investigated in the Caco-2 cell model in order to identify the formulation with the highest mass net flux, which was then administered orally to rats to estimate its availability compared to the pure extract in vivo.



RESULTS AND DISCUSSION

Effect of Phospholipids on the Solubility of 1 and 2. All solubility experiments were carried out with an initial *B. serrata* extract concentration of 5 mg/mL, chosen on the basis of the high dose of this extract used for the treatment of peritumoral edema (3 × 1200 mg/day). Considering an average intake volume of 250 mL (corresponding to a glass of water generally used by the patient to swallow a drug), one dose corresponds to a concentration of 4.8 mg of *B. serrata*/mL. The solubilized fraction of **1** and **2** in water did not exceed 1.16 and 0.2 μg/mL, respectively, indicating poor solubility (Figure 1). In order to better predict the solubility of **1** and **2** in vivo, the solubility of both boswellic acids was also determined in biorelevant media, e.g., FaSSIF and FeSSIF.[27] Much higher solubility could be observed for **1** and **2** in FeSSIF in comparison to FaSSIF. This may be attributed to the 5-fold higher concentration of taurocholate sodium/lecithin[28] and is in line with the observed increase in the absorption of **1**−**6** when taken concomitantly with fatty meals.[11] Whereas the solubility of **1** in FeSSIF was quite reasonable (73.14 μg/mL), that of **2** was still far from a desirable solubility of >60 μg/mL at pH 6.5, which is suggested to enable a reasonable absorption provided that the compounds exhibit good permeability.[29] Complexation of *B. serrata* extract with an equivalent part of phospholipid (Lipoid S100) led to an increased solubility of **1** in FeSSIF (251.71 μg/mL) and FaSSIF (124.03 μg/mL), corresponding to 49.6% and 24.6% of the initial concentration, respectively. The solubility of **2** was also increased compared to the pure extract but to a much lower extent, reaching only 47.95 μg/mL (corresponding to 50% of the initial concentration) in FeSSIF. Complexation of the extract with three parts Lipoid S100 led to a dramatic increase in the solubility of **1** and **2** in water. Obviously, at this extract lipid ratio (1:3 w/w) the lipid concentration is high enough to form vesicles with high loading capacity in aqueous media. On the other hand, the solubility of the 1:3 (w/w) extract−lipid formulation revealed a lower solubility in FeSSIF compared to the 1:1 (w/w) extract−lipid formulation, probably because of the disintegration of

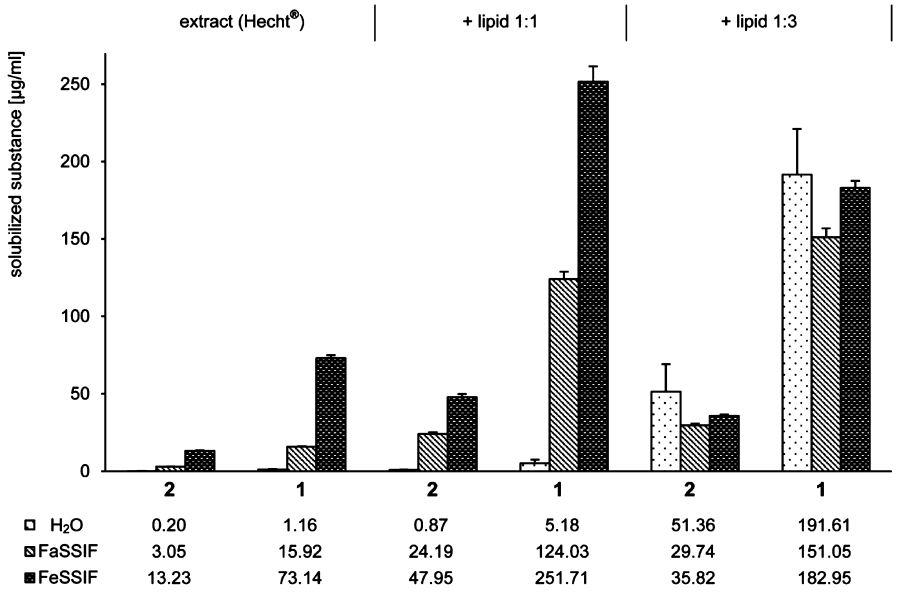


Figure 1. Solubilized fractions of **2**/**1** [μg/mL] after incubation of nonformulated *B. serrata* extract (Hecht), 1:1 (w/w) extract−Lipoid S100, and 1:3 (w/w) extract−Lipoid S100 formulation for 3 h with water, FaSSIF, and FeSSIF, using an initial *B. serrata* extract concentration of 5 mg/mL, corresponding to 96 μg/mL **2** and 504 μg/mL **1**, respectively (n = 3).

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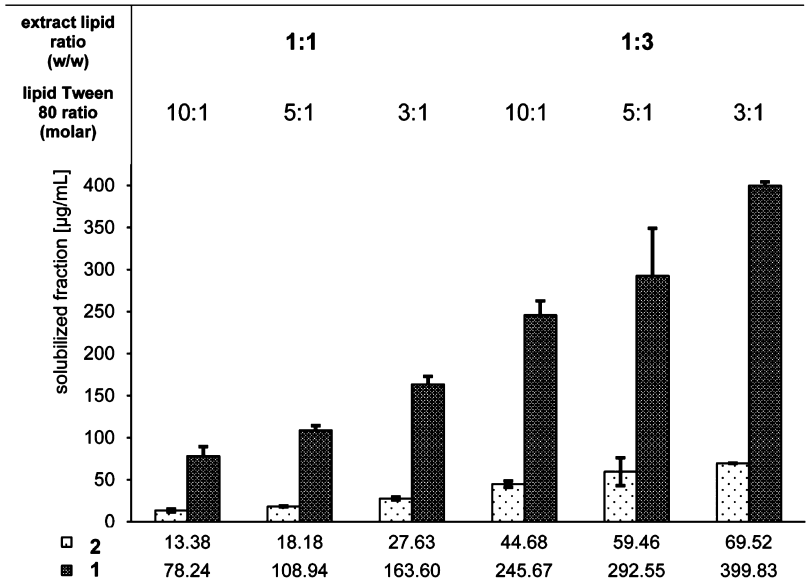

Figure 2. Effect of the addition of Tween 80 on the solubility of the formulation. The bars represent the solubilized fractions of **2** and **1** [μg/mL] at increasing phospholipid and co-surfactant concentrations in modified FaSSIF; initial concentrations of **2** and **1** were 96 and 504 μg/mL, respectively (n = 3).

mixed micelles containing bile salts following the addition of larger amounts of phospholipids.

On the basis of the reported significant increase in the solubility (by 75%) and permeability of structurally similar compounds such as progesterone on preparation of mixed-micellar proliposomal systems,[30] different amounts of Tween 80 (also known as polysorbate 80) were added to the extract–phospholipid complex at both ratios (1:1 and 1:3 extract–lipid). In view of the subsequent transport experiments with Caco-2 cells, the solubility was tested in modified FaSSIF, which was prepared by directly adding the micelle-forming substances (e.g., lecithin and taurocholate sodium) to the HBSS transport medium applied in the Caco-2 cell model.

A constant increase in the solubilized fractions of **1** and **2** was observed with increasing lipid and co-surfactant load, reaching 399.83 μg/mL for **1** and 69.52 μg/mL for **2** at an extract–lipid weight ratio of 1:3 and a lipid–Tween 80 molar ratio of 3:1 (Figure 2). This corresponds to 79.33% and 72.4% of the initial concentrations of **1** and **2**, respectively. In order to keep the fraction of surfactant as low as possible, the formulation with an extract–lipid ratio of 1:3 and a lipid–Tween 80 molar ratio of 5:1 was used for further permeability investigations in the Caco-2 model. This formulation also led to a desirable solubility of 59.46 μg/mL for **2**, promising higher availability in vivo.

Effect of the Extract–Phospholipid Complex on the Permeability of 1 and 2 in the Caco-2 Cell Model. In order to test whether the observed increased solubility of **1** and **2** also results in better absorption, the permeability of the formulation composed of an extract–lipid ratio of 1:3 and a molar lipid–Tween 80 ratio of 5:1 was compared with that of the nonformulated extract, the extract–lipid complex (1:3 w/w), and the extract–lipid complex (1:3 w/w) using vitamin E-TPGS as an alternative surfactant in the Caco-2 model. The latter is used often as a solubilizing agent and an availability enhancer in lipid-based formulations. In general, transport experiments with lipophilic drugs are often hampered by their low solubility and accumulation in Caco-2 cells, leading to poor reproducibility and low mass balance. These drawbacks may be overcome by providing sink conditions. This implies the provision of a sufficiently diluted system in the receiver compartment, so that the dissolution of **1**–**6** is not impeded by saturation effects. For that reason 4% BSA was added to HBSS buffer in the basolateral receiver compartment in order to provide the necessary driving force similar to in vivo sink conditions, where the serosa side was perfused with blood containing about 4% albumin.[31] By this means the mass balance of **1** and **2** could be improved from 40% to 60% in preliminary permeability tests with pure HBSS buffer (data not shown) to values around 100% in the present study. For further adaptation of the Caco-2 model to physiological conditions, FaSSIF, simulating the gastrointestinal content at the site of absorption in the fasted state, was used as donor fluid on the apical side. For this purpose, micelle-forming substances (e.g., lecithin and taurocholate sodium) were added directly to HBSS transport medium [+ 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 6.5], yielding modified FaSSIF. In contrast to other surfactants, up to 150 mg/mL phospholipids organized in liposomal structures may be applied on Caco-2 cells without disturbing the membrane integrity.[29,32] Since the solubilizing capacity of modified FaSSIF (16.99 μg/mL for **1**) was found to be comparable with FaSSIF (15.92 μg/mL) and pure HBSS containing 4% BSA (17.47 μg/mL) after 3 h of incubation at 37 °C, modified FaSSIF was applied at the donor side in all transport experiments for simplification.

As seen in Table 1, complexation with phospholipids in formulation B (extract–lipid, 1:3 w/w) increased the net flux of **1** (8 times) and **2** (15 times) in comparison with the non-formulated extract. On the other hand, formulation C, composed of an extract–lipid ratio of 1:3 and a lipid–Tween 80 molar ratio of 5:1, showing the highest solubility in the preliminary tests, led to a complete retention of **1** and **2** in the donor compartment (Table 1).

These results indicate that an increase in solubilizing capacity is not necessarily associated with improved permeability. As the drug-release out of a vesicular carrier system displays the limiting step, the affinity to the surfactant should be as low as possible. A significant improvement in mass net flux for **1** (20 times) and **2** (29 times) compared to the nonformulated extract could be

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Table 1. Caco-2 Cell Transports with Nonformulated Extract in Comparison with the Extract−Phospholipid Complex with and without Co-surfactant Using an Initial Extract Concentration of 5 mg/mL *B. serrata* Extract Corresponding to 504 μg/mL (1) and 96 μg/mL (2)[a]

formulation		initial conc *t*0 [μg/mL]	mass flux [ng·cm^{-2}·min^{-1}]	mass balance [%]
A: nonformulated extract (reference)	1	3.05	3.07 ± 0.09	100.60
	2	0.39	0.24 ± 0.04	95.17
B: extract−lipid 1:3 (w/w)	1	108.84	24.02 ± 2.08	96.03
	2	20.97	3.59 ± 0.37	96.37
C: extract−lipid 1:3 (w/w) + Tween 80 (lipid−Tween 80 molar ratio 5:1)	1	233.40	<LOD[b]	101.70
	2	46.06	<LOD	104.12
D: extract−lipid−vit E-TPGS 1:2:1 (w/w/w)	1	397.19	59.95 ± 3.04	99.85
	2	71.22	6.84 ± 0.22	98.03

[a]Values are means of three experiments ± SD. [b]<LOD: below limit of detection.

achieved by replacing Tween 80 with vitamin E-TPGS and by the reduction of the lipid content to a 2-fold weight ratio in comparison to the extract (formulation D). Further reduction of the lipid fraction, resulting in an equally weighted extract−lipid−vit E-TPGS ratio (formulation G, Table 2), revealed comparable

Table 2. Caco-2 Cell Transports Carried out with Equally Weighted Extract/Lipid/Cosurfactant Formulations Using Different Co-surfactants and a Fixed Extract Concentration of 5 mg/mL Corresponding to 504 μg/mL (1) and 96 μg/mL (2)[a]

		initial conc *t*0 [μg/mL]	mass flux [ng·cm^{-2}·min^{-1}]	mass balance [%]
E: extract−lipid (1:1 w/w)	1	175.30	49.87 ± 2.04	93.94
	2	29.99	5.11 ± 0.12	88.28
F: extract−lipid−Tween 80 (1:1:1 w/w/w)	1	316.91	58.76 ± 0.45	96.30
	2	39.40	4.56 ± 0.33	93.27
G: extract−lipid−vitamin E-TPGS (1:1:1 w/w/w)	1	395.20	53.52 ± 1.31	100.68
	2	70.31	6.18 ± 2.15	98.78
H: extract−lipid−gelucire 44/14 (1:1:1 w/w/w)	1	380.27	49.06 ± 1.44	91.97
	2	67.33	5.71 ± 0.12	90.71
I: extract−lipid−pluronic f68 (1:1:1 w/w/w)	1	215.49	69.66 ± 0.27	90.14
	2	36.83	9.59 ± 0.14	87.81
K: extract−lipid−pluronic f127 (1:1:1 w/w/w)	1	395.04	82.34 ± 6.24	99.90
	2	65.34	10.17 ± 0.76	100.70

[a]Values are means ± SD of three experiments.

mass net flux for **1** (53.52 ng·cm^{-2}·min^{-1}) and **2** (6.18 ng·cm^{-2}·min^{-1}). As a lower surfactant load is generally favored in the light of clinical applicability, the equally weighted ratio of extract/lipid/co-surfactant was kept constant in further transport experiments replacing only the co-surfactant. The highest mass net flux (82.34 for **1** and 10.17 ng·cm^{-2}·min^{-1} for **2**) was determined for formulation K, containing pluronic f127 (also known as Lutrol f127 or poloxamer 407, Table 2).

This formulation led also to the highest solubility for **1** (395.04 μg/mL) and **2** (65.34 μg/mL) compared to the other formulations. The mass flux of formulation I containing pluronic f68, representing another block copolymer of lower molecular weight, remained slightly beyond that of pluronic f127, indicating the general suitability of this substance class.

On the basis of these findings, formulation K, composed of extract−lipid−pluronic f127 (1:1:1 w/w/w), gave the best results. In order to exclude any influence of the transport medium on the permeability, taking the varying fractions of micelles in FaSSIF and FeSSIF into consideration, the P_{app} values for formulation K were also determined in modified FeSSIF and HBSS in comparison to modified FaSSIF. The use of high bile salt concentrations, as in the case of modified FeSSIF (15 mM), led to a strong decrease of the initial TEER value beyond the acceptable level of 300 Ω·cm^2 (Table 3), while modified FaSSIF

Table 3. Overview of the P_{app} Values Determined for 1 and 2 Using Formulation K in Different Apical Media: Modified FaSSIF, Modified FeSSIF, and Pure HBSS Buffer[a]

		P_{app} [cm/s]	TEER before/after [Ω·cm^2]
mod. FaSSIF	1	3.7 × 10^{-6} ± 132.0 × 10^{-9}	458 ± 22.5/
	2	3.1 × 10^{-6} ± 114.6 × 10^{-9}	337 ± 2.5
mod. FeSSIF	1	3.2 × 10^{-6} ± 879.4 × 10^{-9}	436 ± 5.0/
	2	2.8 × 10^{-6} ± 526.7 × 10^{-9}	114 ± 11.9
HBSS	1	2.9 × 10^{-6} ± 21.1 × 10^{-9}	441 ± 7.2/
	2	2.5 × 10^{-6} ± 65.1 × 10^{-9}	409 ± 46.2

[a]Values are means ± SD of n = 3 experiments.

and pure HBSS fulfilled the specifications. Nevertheless, comparable P_{app} values were obtained for **1** and **2** in the different media used, confirming the robustness and low influence of the donor medium on permeability and drug release from the formulation. The fact that the decrease of tight junction integrity did not manifest in higher P_{app} values is an indication of the passive transcellular transport of both compounds **1** and **2**.

Finally, formulation K was mixed at a 1:1 weight ratio with microcrystalline cellulose in order to generate a dispensable free-flowing powder that can be further processed in the form of tablets or capsules. In order to ensure that the addition of microcrystalline cellulose had no negative effects on solubility, the solubility of the powdered formulation K was determined in physiologically relevant media. The solubility of both **1** and **2** using the powdered formulation was increased 28 times in pure water, 54 times in simulated gastric fluid (SGF_{sp}), 37 times in FaSSIF, and 10 times in FeSSIF, when compared to the nonformulated extract (data not shown).

On the basis of these results, around 70% of **1** and **2** present in high *B. serrata* extract doses applied in the treatment of peritumoral edema was dissolved within 85 min at a pH value of 6.5 (FaSSIF) and around 90% was dissolved at pH 5.0 (FeSSIF), indicating the suitability of the present formulation to be incorporated in capsules for oral administration.

In order to verify these in vitro results and to prove the suitability of formulation K in vivo, its availability was compared with that of the nonformulated extract in rats following oral administration.

Effect of the Extract−Phospholipid Complex on the Availability of Boswellic Acids in Rats. No physical signs of distress were noted in any of the rats during the study. The concentrations of all six major boswellic acid derivatives present in *B. serrata* extract (**1**−**6**) were determined in rat plasma and brain using a newly developed LC-MS method.[33]

As seen in Figure 3, the plasma concentrations of all boswellic acids determined after the administration of formulation K exceeded the levels of the reference nonformulated extract at all sampling points. Formulation K is characterized by a more rapid

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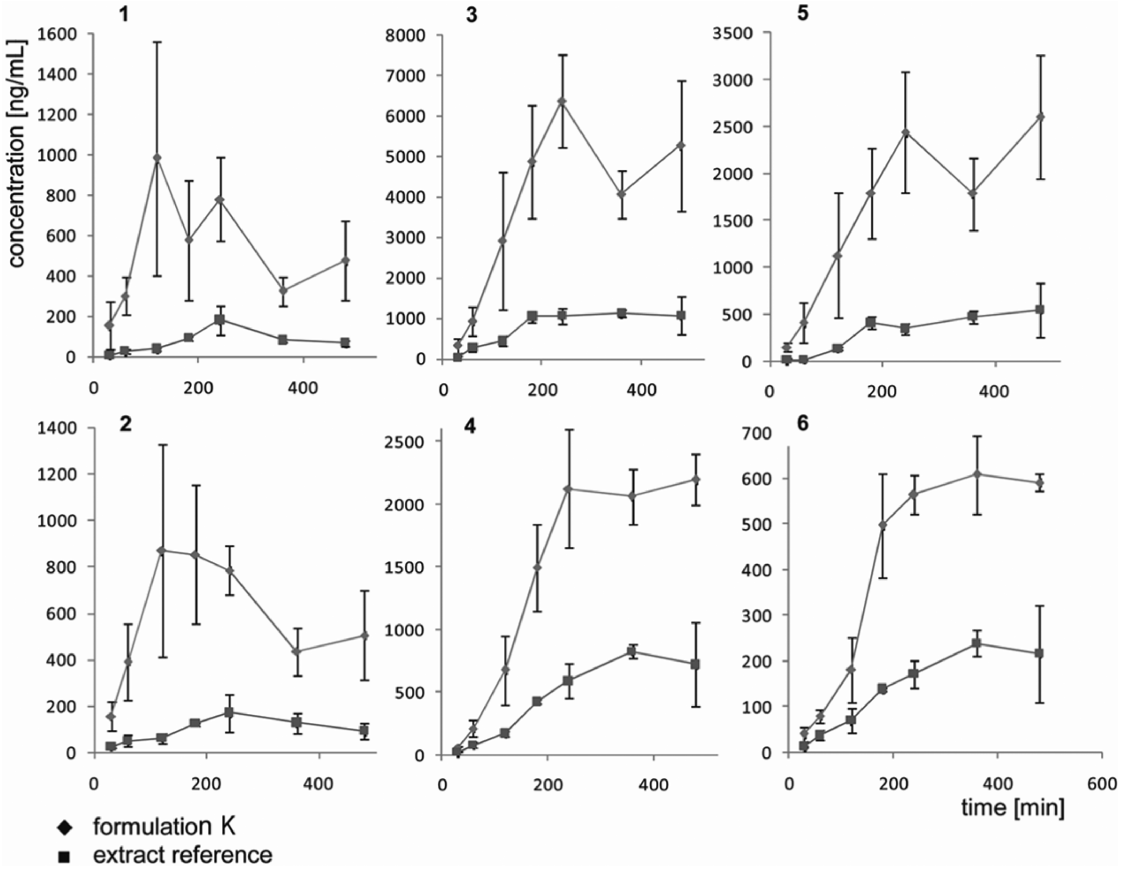

Figure 3. Mean plasma profiles of boswellic acids **1**–**6** after oral administration of 240 mg/kg *B. serrata* reference extract and formulation K (corresponding to 240 mg/kg *B. serrata* extract) over 8 h. Values are means ± SD of three rats.

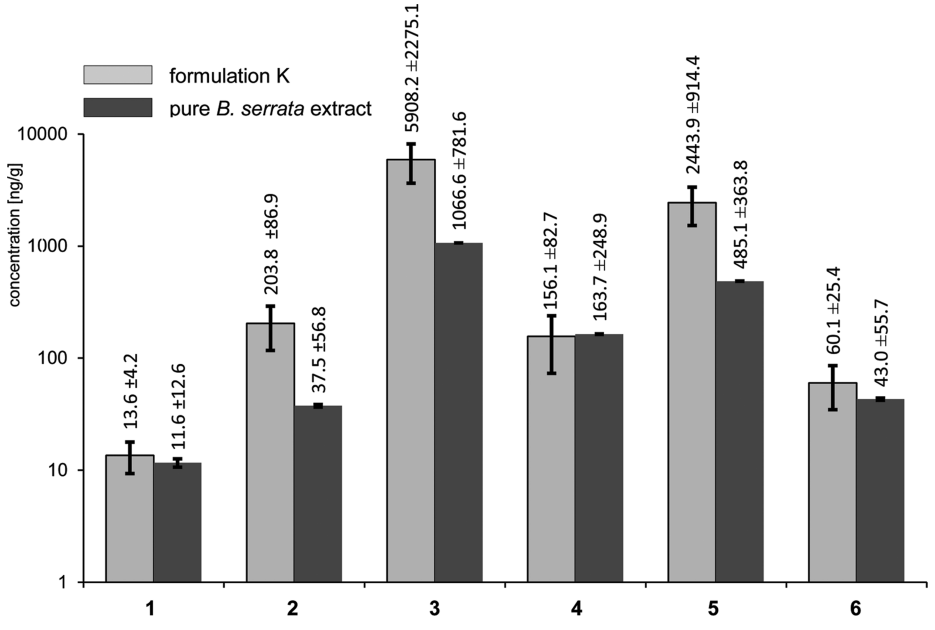

Figure 4. Mean concentrations of boswellic acids (**1**–**6** ng/g) in brain after 8 h of the oral administration of 240 mg/kg pure *B. serrata* extract and formulation K (corresponding to 240 mg/kg *B. serrata* extract). Values are means ± SD of six rats. The *y*-axis is presented in logarithmic scale.

rise in the plasma levels of **1** and **2**, reaching 26 and 14 times higher concentrations than the reference extract after 2 h. Both of these compounds showed another maximum after 8 h and maintained 6-fold higher levels compared to the reference nonformulated extract.

The sharper rise in plasma levels was also observed with **3**–**5** from formulation K, leading to a first maximum after 4 h followed by another after 6 h. In the case of **6**, a delayed maximum after 6 h was obtained, suggesting a more sustained release. Taken together, formulation K contributed up to a 26-fold increase in

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the concentrations of boswellic acids in plasma, confirming the higher mass net flux determined in the Caco-2 model. Furthermore, this is the first formulation leading to concentrations of **2** in plasma (868.8 ng/mL; i.e., 1.7 μM) corresponding to the pharmacologically active concentrations for 5-LO inhibition (1.5 μM), as determined by in vitro assays.[3] Also, the plasma levels of **3** exceeded the IC_{50} value of 5 μM for the inhibition of mPGES-1 by 14 times.[3] Likewise, analysis of brain samples following the administration of formulation K revealed higher levels for most boswellic acids compared to the reference nonformulated extract (Figure 4).

Thus, a 1.2-fold increase in the level of **6** and up to 5-fold higher levels for **2**, **3**, and **5** were obtained following the administration of formulation K, whereas the brain levels of **1** and **4** were comparable with those of the pure extract. The highest brain level was obtained for **3** (on average 5908 ng/g; i.e., 13 μmol/g), corresponding to the pharmacologically active concentrations determined for the inhibition of mPGES-1 in vitro. Brain/plasma ratios greater than 1 were calculated for **2**, **3**, and **5** (as 2.2, 5.5, and 4.4) 8 h after the oral administration of formulation K. In contrast, the administration of the nonformulated *B. serrata* extract revealed only low brain-plasma ratios for **2**, **3**, and **5**, of 0.07, 0.2, and 0.18, respectively. These results show that the increase in plasma levels is clearly accompanied with a better availability of **2**, **3**, and **5** in the brain. This is of potential importance for clinical practice, as higher levels of **1**−**6** in the brain are needed to achieve better results in the treatment of peritumoral edema, especially when it is considered that until now no boswellic acids could be detected in brain tumors of patients who had taken *B. serrata* extract for several weeks before their operation.

In conclusion, in the current study a formulation has been developed, consisting of *B. serrata* extract−Lipoid S100−pluronic f127 (1:1:1 w/w/w), resulting in an improved boswellic acid solubility, which consequently led to improved absorption in the Caco-2 model. The in vitro results obtained could be confirmed in vivo. Thus, up to 25 times higher plasma levels and 5-fold higher brain levels could be observed for **1**−**6** in rats following oral administration of the formulation developed in comparison to a nonformulated *B. serrata* extract, resulting in concentrations for **2** and **3** corresponding to the pharmacologically active concentrations determined in vitro. This observed increase in the availability of boswellic acids encourages further pharmacokinetic and clinical research on this promising anti-inflammatory phytomedicine. Moreover, the present study shows that increasing the solubility of poorly soluble but well permeable lipophilic herbal ingredients is a promising approach to enhancing absorption.

EXPERIMENTAL SECTION

General Experimental Procedures. Lipoid S100 (aka PCS100), containing phosphatidylcholine (≥94%) with an average molecular weight of 786 g/mol (lysophosphatidylcholine ≤3%), was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Boswellic acids (**1**−**6**, purity >99%) were obtained from Phytoplan Diehm & Neuberger GmbH (Heidelberg, Germany). H15 capsules containing 350 mg of *B. serrata* extract (Hecht-Pharma GmbH, Stinstedt, Germany), used for the solubility and transport experiments, were purchased from Anzag (Frankfurt am Main, Germany), and nonformulated *B. serrata* extract, applied in the rat study was kindly donated by Indena SpA (Milan, Italy). Propranolol hydrochloride was obtained from Fagron GmbH & Co. KG (Barsbüttel, Germany), while pluronic f68/127 (aka Lutrol) and gelucire 44/14 were kind gifts from BASF ChemTrade GmbH (Burgbernheim, Germany) and Gattefossé (Saint-Priest, France), respectively. Microcrystalline cellulose, HEPES, vitamin E-TPGS, fluorescein sodium, and Tween 80 were purchased from Sigma-Aldrich (Steinheim, Germany), and ammonium formate (p.a.) was provided by VWR (Leuven, Belgium). Phares SIF powder was obtained from Phares AG (Muttenz, Switzerland), and albumin bovine fraction V (BSA, standard grade, M_r ~67.000) was obtained by Serva Electrophoresis GmbH (Heidelberg, Germany). All solvents, comprising methanol, tetrahydrofuran, ethyl acetate, and *n*-hexane (analytical grade or better), were purchased from Roth GmbH (Karlsruhe, Germany) and Merck (Darmstadt, Germany).

Preparation of *B. serrata*−Phospholipid (±Co-surfactant) Complexes. *B. serrata*−phospholipid complexes for solubility tests and transport studies were prepared according to the solvent evaporation method. For this purpose, phospholipid/surfactants were dissolved in methanol to yield a final stock solution concentration of 400 mg/mL, and the *B. serrata* stock solution contained finally 37.10 mg/mL of the whole extract. Respective aliquots of methanolic *B. serrata* (containing 75 mg of the extract) and phospholipid/surfactant stock solutions (containing for example 75 mg for 1:1 weight ratio) were transferred into a round-bottomed flask and dried under a stream of nitrogen. The resulting film was further treated with high vacuum (at 40 °C) until complete dryness, followed by rehydration with 15 mL of the respective test medium to yield an initial extract concentration of 5 mg/mL. Prior to Caco-2 investigations, nonformulated and complexed *B. serrata* extracts were incubated with 15 mL of modified FaSSIF for 1.5 h in a shaking water bath (Julabo SW22, Seelbach, Germany; 37 °C, 120 rpm), centrifuged (4000 rpm, 25 °C) to remove the unsolved fraction, and then applied to the cells.

For the preparation of a free-flowing powder, 1.5 g of *B. serrata* extract (H15 capsules, Hecht), 1.5 g of Lipoid S100, and 1.5 g of pluronic f127 were weighed in a round-bottomed flask. Then, 20 mL of methanol was added, and the flask was rotated for 10 min at 40 °C (70 rpm). Subsequently, 4.5 g of microcrystalline cellulose was added, and the solvent was slowly removed under vacuum in a rotary evaporator for 1 h (40 °C, 70 rpm). Afterward, the resulting formulation was treated in a high-vacuum drying cabinet (45 °C) for 24 h until complete dryness.

In the case of the rat study, 300 mg of the nonformulated extract, Lipoid S100, and pluronic f127, respectively, were directly weighed into test tubes and rehydrated with 5 mL of H_2O under vigorous shaking (with short and mild heating at <45 °C, if necessary) to yield a final concentration of 60 mg/mL *B. serrata* extract.

Preparation of Simulated Intestinal Fluids. Phares SIF powder, a combination product containing taurocholate sodium and lecithin in a 4:1 molar ratio, was used for the preparation of the simulated intestinal fluids, FaSSIF and FeSSIF, according to the manufacturer's instructions. FaSSIF consists of 3 mM taurocholate sodium and 0.75 mM phosphatidylcholine in dihydrogenphosphate sodium buffer (28.66 mM, pH 6.5), while FeSSIF contains 15 mM sodium taurocholate and 3.75 mM phosphatidylcholine in acetic acid buffer (144 mM) adjusted to pH 5.0. Appropriate amounts of SIF powder were diluted with the corresponding buffer and shaken at room temperature until complete solution. The prepared solutions were stored under light protection at room temperature for 2 h to allow micelle formation and were used within 48 h. Simulated gastric fluid was prepared according to USP 34 (no pepsin was used) by dissolving 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and sufficient water to yield 1000 mL.

Solubility Tests. Standardized Hecht H15 capsules used for solubility tests were quantified for the content of **1** and **2**. For this purpose, a methanol stock solution was prepared by transferring the contents of 16 capsules (350 mg BSE/capsule) into a 100 mL volumetric flask and adding 50 mL of methanol. After 10 min of ultrasonic treatment, the flask was filled to the required volume and the extract was filtrated through a folded filter, in order to remove interfering insoluble substances. Afterward, the concentrations of **1** and **2** were determined by HPLC, demonstrating a content of 3.7 mg/mL for **1** and 0.6 mg/mL for **2**.

For the solubility tests of the extract−phospholipid complexes, 15.0 mL of the solvents (H_2O, FaSSIF, FeSSIF) was added to the dried extract film in triplicate to yield a final extract concentration of 5 mg/mL, corresponding to 504 μg/mL **1** and 96 μg/mL **2**. As a reference, 75 mg

of the nonformulated H15 extract (powder form) was directly added to the respective solvent. Solubility tests were carried out in a shaking water bath (Julabo SW22, Seelbach, Germany) at 37 °C and 120 rpm for a maximum of 3 h. Aliquots of 0.5 mL were collected after 3 h and centrifuged at 4000 rpm (25 °C). Finally, 100 μL of the supernatant was diluted to 1 mL with methanol and analyzed by HPLC.

Solubility tests of the powdered formulation were carried out by adding the respective amount of the formulation into a conical flask containing 15 mL of the respective medium (H_2O, SGF_{sp}, FaSSIF, FeSSIF), to yield a final extract concentration of 5 mg/mL. Weighed samples were incubated in triplicate for 3 h in a shaking water bath (37 °C, 120 rpm). For HPLC analysis aliquots of 0.5 mL were taken after 180 min and centrifuged (4000 rpm, 25 °C), and 100 μL of the supernatant was further diluted with methanol to get a final ratio of 9:1 (methanol−sample).

Transport Experiments. Differentiated Caco-2 cells of passages 24−36, purchased from the American Type Culture Collection (ATCC, Maryland, USA), were used for transport studies and plated at a density of 6.5×10^4 cells per cm^2 on 12-well Transwell plates (1.2 cm^2 polycarbonate membrane, 0.4 μm pore size, donor volume 0.5 mL, receiver volume 1.5 mL, Corning, NY, USA). The cells were cultivated in an atmosphere consisting of 90% relative humidity, with 10% CO_2, and at 37 °C. Prior to analysis, they were grown in Dulbecco's modified Eagle's medium containing 25 mM glucose supplemented with 10% fetal calf serum, 1% nonessential amino acids, and gentamycin (0.1 mg/mL). All media were purchased from Biochrom AG, Berlin, Germany. In cultivation, cells were passaged once a week to avoid differentiation, and the culture medium was changed three times a week. Then, cells were sown on transport plates and left for differentiation and formation of a confluent monolayer between the 21st and 28th day. Transport experiments were conducted in an apical→basolateral direction (AB). Plates were shaken (120 rpm) and kept at a constant temperature (37 °C). Hank's buffered salt solution (HBSS + 10 mM HEPES) was used for the transport experiments, which was either applied in pure form or supplemented with different additives [e.g., taurocholate sodium/lecithin (Phares SIF) on the apical side and 4% BSA on the basolateral side] across a pH gradient (apical, pH 6.5; basolateral, pH 7.4), as described in the respective section. Permeability and integrity of every cell passage were monitored by concomitant transport of propranolol hydrochloride (positive control, transcellular marker) and fluorescein sodium (negative control, paracellular marker) and by the control of the TEER value (transepithelial electrical resistance) just before and after the experiment. Transport results were evaluated only if the following internal specifications were fulfilled: P_{app} of propranolol hydrochloride $>10 \times 10^{-6}$ [cm/s], P_{app} of fluorescein sodium $<1 \times 10^{-6}$ [cm/s]; TEER >300 [Ω*cm^2]; mass balance of analyte 70−120% (donor + receiver fraction after transport of the initial concentration t_0). The mass flux was calculated according to the following formula:

$$J = \frac{m}{At}[\mu g\ cm^{-2}\ h^{-1}]$$

where J = mass flux of a compound, m = mass of a compound, A = surface area of the cell monolayer (cm^2), and t = time.

For the determination of the initial concentration (t_0), 100 μL aliquots were taken from the transport solution to be tested, diluted with 900 μL of methanol, and analyzed by HPLC. During transport in the AB direction (absorptive direction), the whole basolateral medium (1.5 mL) was removed at fixed time points and replaced by fresh buffer/transport medium. Then, 750 μL of the removed basolateral medium was transferred into a test tube, mixed with 4 mL of extraction medium (THF−ethyl acetate−*n*-hexane−2-propanol, 320:320:320:30), and shaken for 10 min on a flatbed shaker. After 5 min of phase separation, 3 mL of the upper organic phase was collected and the organic solvent was evaporated under a stream of nitrogen. Substances were resolved in 250 μL of MeOH−H_2O (9:1), vortexed, treated in an ultrasonic water bath for 5 min, and analyzed by HPLC. For the determination of the concentration on the donor side at the end of the transport experiments, 100 μL of the donor volume was diluted with 900 μL of methanol and also analyzed by HPLC.

For the determination of P_{app} values, transports were carried out over 270 min, and the basolateral medium was exchanged and analyzed after 90, 180, and 270 min in triplicate. At every sampling point, the fraction of transported substance remained below 10% of the initial concentration, so that a sufficient concentration gradient and sink conditions were guaranteed over all transport experiments.

Analysis of 11-Keto-β-boswellic Acid (1) and 3-Acetyl-11-keto-β-boswellic Acid (2) by HPLC. For the determination of compounds **1** and **2**, an existing bioanalytical method was modified and expanded.[8] Briefly, the system was composed of a LiChrospher 100, RP-18 Merck column, 5 μm (125 × 4 mm), and a mobile phase consisting of (A) water−methanol−orthophosphoric acid (85%) (90:9.5:0.5 v/v/v) and (B) methanol−acetonitrile−water−orthophosphoric acid (85%) (55:40:4.5:0.5 v/v/v). The gradient was kept constant at 90% B for 11 min and was then changed to 100% B in 4 min before re-equilibration was initiated for 15 min until the next injection. The flow rate was set to 1 mL/min, the injection volume was 75 μL, and UV detection was carried out at 250 nm. Calibration standards, covering a concentration range of 0.2−32 μg/mL, were prepared from methanol working solutions of pure **1** and **2**. The solutions were processed and diluted with a final diluent consisting of methanol−water (9:1). The analysis was carried out on an Agilent Technologies 1200 Series chromatographic system equipped with Chemstation software and a Merck-Hitachi LaChrom system (Merck, Darmstadt).

Rat Study. Experiments were carried out according to the guidelines of the German Protection of Animal Act (Deutsches Tierschutzgesetz, BGBI 1998). Test solutions were administered orally by gavage via a pharyngeal tube to achieve a final dose of 240 mg of *B. serrata* extract/kg. Blood samples for plasma analysis were collected from the retrobulbar venous plexus of the anesthetized animals after defined time points (0.5, 1, 2, 3, 4, 6, and 8 h) in lithium−heparin tubes, centrifuged, and stored at −80 °C. After 8 h, rats were dissected, and the brain was isolated, washed with 5 mM Tris-HCl buffer (pH 7.4), and weighed. The whole brain was homogenized in Tris-HCl buffer (1 mL of buffer/100 mg of brain) and analyzed by LC-MS.

Analysis of Plasma and Brain Samples by LC-MS. Briefly, a Hypersil BDS RP C_{18} column (100 × 4 mm, 3 μm, Thermo Scientific) in combination with a Gemini C_{18} security guard cartridge (4 × 3 mm, Phenomenex, Germany) was used for chromatography on an Agilent 1200 series (Agilent Technologies, Waldbronn, Germany). The gradient program, starting with 90% mobile phase A (methanol−water, 90:10, 400 mg/L ammonium formate) and 10% mobile phase B (methanol−water, 80:20, 400 mg/L ammonium formate), was changed to 100% mobile phase A within 20 min. This solvent was held constant for a further 14 min, and then the gradient was changed to the starting combination within 1 min, resulting in a final run time of 35 min. The flow rate was set to 0.4 mL/min, the column oven was held at a constant temperature of 35 °C, and the autosampler was kept at room temperature. MS analysis was performed in the negative single ion mode on an Agilent Triple Quadrupole LC/MS 6410 series (Agilent Technologies, Waldbronn, Germany) using an electrospray ionization source and a dwell time of 200 ms.

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Notes

The authors declare no competing financial interest.

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