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Lessons Learned from Herbal Medicinal Products: The Example of St. John's Wort[⊥]

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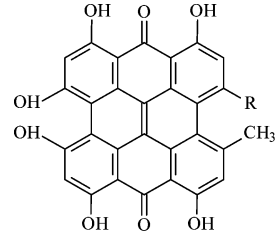
The example of St. John's wort offers convincing evidence for the concept that modern methods of pharmacological and phytochemical research are effective in advancing the development of traditional herbal remedies. As a consequence of these efforts, it is known today that several compounds from different structural groups and with different mechanisms of action seem to be responsible for the observed antidepressant efficacy of St. John's Wort. Co-effectors in the extract improve the bioavailability of active constituents such as hypericin (**1**) (pharmacokinetic synergy). Unwanted side effects are preventable without remarkable loss of activity when the responsible constituent(s) are carefully removed during the extraction process, as demonstrated for hyperforin (**3**), which is responsible for the induction of cytochrome P450 (CYP)-metabolizing enzymes (CYP3A4, in particular). On the basis of our findings, it is likely that positive interactions between single compounds occur more frequently in traditionally used herbal preparations than is known presently.

Introduction

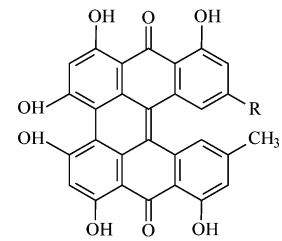
Depression is a mental illness characterized by symptoms such as anxiety, hopelessness, loss of energy and appetite, insomnia, and thoughts of death. It is one of the leading causes of disability worldwide, with approximately 120 million persons affected in industrial countries.[1] *Hypericum perforatum* L. (Clusiaceae), commonly known as St. John's wort (SJW), is used in many countries for the treatment of mild to moderate forms of depression.[2,3] The crude drug of St. John's Wort consists of the upper third of the flowering parts of *H. perforatum*, and usually hydroethanolic extracts are prepared to produce solid or liquid dosage forms that are on the market as botanical dietary supplements. More than 50 controlled clinical studies in depressed patients have been performed with a dosage of 600−900 mg of extract per day (equivalent to approximately 3 to 5 g of crude drug material), showing efficacy equal to standard antidepressants including the modern selective serotonin reuptake inhibitors (SSRIs).[2] The present paper summarizes the results of our work on active constituents in St. John's Wort, offering general conclusions that can be drawn from these findings and finally discussing the data in view of present knowledge about this important herbal product.

From a phytochemical point of view, *H. perforatum* represents one of the best-investigated medicinal plants; overviews about the phytochemical composition of the crude drug material as well as the hydroalcoholic extract have been published.[4−6] The naphthodianthrones,[7] hypericin (**1**) and pseudohypericin (**2**), and related compounds (e.g., their proto derivatives **1a** and **2a**) have been identified as typical constituents of the genus *Hypericum* at amounts of 0.1−0.5% in extracts of the crude drug. In *H. perforatum*, the ratio of **1** to **2** can reach 1:10. The light-sensitive and unstable prenylated polyketide hyperforin (**3**)[8] and its derivatives were first isolated and identified by Russian workers;[9,10] they reach up to 5% in extracts. One of the degradation products of the hyperforins is 2-methyl-3-buten-2-ol,[11] which acts as a sedative in higher doses. Several flavonol glycosides (3−12%), mainly quercetin (**4**) derivatives, with the main representatives hyperoside (quercetin 3-*O*-galactoside, **5**) and rutin (quercetin 3-*O*-rutinoside, **9**) and minor compounds such as quercitrin (quercetin 3-*O*-rhamnoside, **6**), isoquercitrin (quercetin 3-*O*-glucoside, **7**), and miquelianin (quercetin 3-*O*-glucuronide, **8**) were identified,[12] but kaempferol glycosides and flavanol derivatives were rarely found.[13] Biflavones (0.3−0.7%, **13**, **14**) are present in *H. perforatum* extracts such as I3,II8-biapigenin (**13**), which is about 10-fold higher in concentration than amentoflavone (I3′,II8-biapigenin, **14**); nevertheless amentoflavone is often used in preclinical studies because of its commercial availability. St. John's Wort extracts are rich in condensed tannins (up to 15%), such as oligomeric procyanidins (**11** and **12**); the monomeric constituents catechin and epicatechin are also present.[14] Xanthones, in particular kielcorin (**15**), 1,3,6,7-tetrahydroxyxanthone (**16**), and mangiferin (**17**), are present at higher concentrations in the roots, whereas the upper parts contain **16** at 0.0004% and traces of **17** in the dried material (for review: refs 5 and 12). Traces of amino acids and a few essential oil components present in the oil glands have been isolated from the above-ground parts of the plant. Recently, in addition to the main components mentioned above, some plant acids and the carbohydrate fraction of an extract have been investigated, the latter found to be up to 25%.[15] In summary, known bioactive compounds in *H. perforatum* represent approximately 60−70% of the known phytochemical constituents.[15] However, 30−40% of the compounds

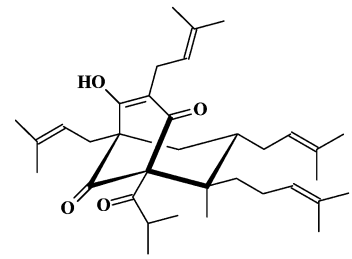
Chart 1



R = CH_3 hypericin (**1**)
R = CH_2OH pseudohypericin (**2**)



proto-derivatives (**1a**, **2a**)



hyperforin (**3**)

[⊥] Adapted from the Varro E. (Tip) Tyler Award for Botanical Research address of A.N. at the 50th Meeting of the American Society of Pharmacognosy, June 27−July 1, 2009, Honolulu, HI.

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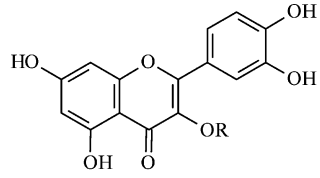
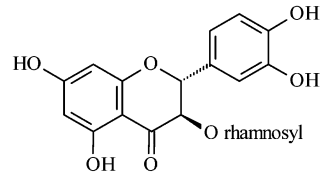
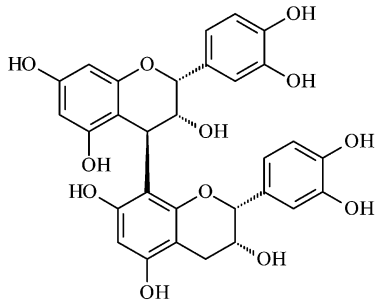
Chart 2



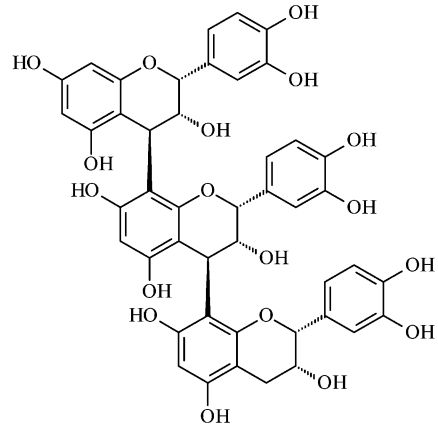
R = H quercetin (**4**)
R = galactosyl hyperoside (**5**)
R = rhamnosyl quercitrin (**6**)
R = glucosyl isoquercitrin (**7**)
R = glucuronosyl miquelianin (**8**)
R = rhamnosyl-glucosyl rutin (**9**)



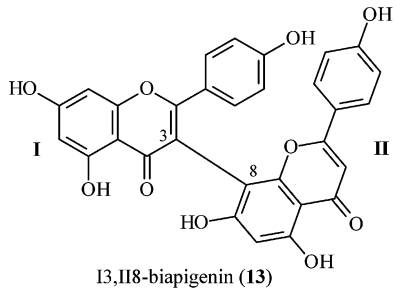
astilbin (**10**)



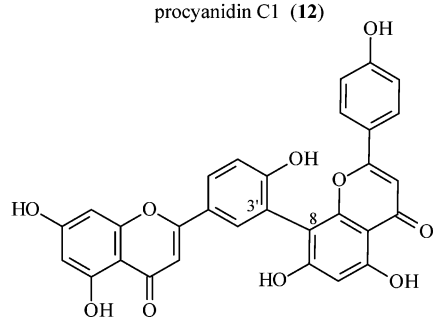
procyanidin B2 (**11**)



procyanidin C1 (**12**)

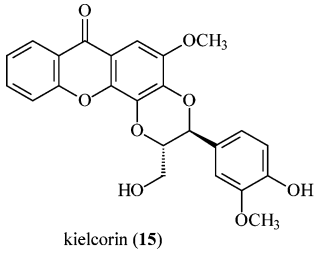


I3,II8-biapigenin (**13**)

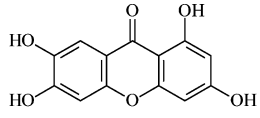


I3', II8-biapigenin (**14**, amentoflavone)

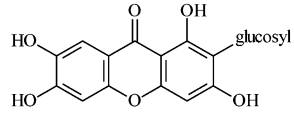
Chart 3



kielcorin (**15**)



1,3,6,7-tetrahydroxyxanthone (**16**)



mangiferin (**17**)

present in *H. perforatum* extracts are as yet not structurally assigned, and among these could be further constituents directly or indirectly contributing to the overall clinical efficacy.

When conducting research on active constituents of herbal products, the question may arise as to why such complicated and often time-consuming work is necessary, especially since from a therapist's standpoint efficacy seems to be more important than the search for active compounds. However, from a pharmaceutical point of view, identification of the active constituents is highly important in order to ensure pharmaceutical and thereby therapeutic quality of the crude drug and its extracts; to a lesser extent the identified active compounds may be used as leads for the development of highly active synthetic drugs. Knowledge of active compounds also helps to optimize the crude drug material, e.g., by selection and breeding. Once the active constituents are known, further investigations on the mechanism(s) of action can be performed that are more straightforward with pure isolates than with the entire herbal extract. Finally, not only active constituents of an herbal preparation should be known but also its entire phytochemistry, because interactions between constituents are possible in a positive and a negative manner, as will be discussed in further detail below.

Active Constituents toward Depression in *H. perforatum*. Initial in vitro investigations on constituents responsible for antidepressant activity of *H. perforatum* indicated hypericin (**1**) to be an inhibitor of monoamine oxidase (MAO) enzymes.[16] However, the hypericin sample was later shown to have been impure, containing at least 20% of other constituents of the extracts, among these flavonoids. In fact, the MAO inhibitory effects of hypericin could not be confirmed in subsequent studies.[17] Amentoflavone (**14**) was shown to have GABAergic activity;[18] however, this compound is too low in concentration to be responsible for therapeutic activity of *H. perforatum*. Unfortunately, the more highly concentrated structurally related I3,II8-biapigenin (**13**) was not tested. 1,3,6,7-Tetrahydroxyxanthone (**16**) and some flavonoids were detected as inhibitors of MAO-A and MAO-B;[13] however, as mentioned earlier, the amount of xanthone in *H. perforatum* extracts is too low to explain the observed in vivo antidepressant activity. Later, Chatterjee, Mueller, and co-workers showed that hyperforin (**3**) in vitro is capable of inhibiting the reuptake of serotonin (5-HT), norepinephrine (NE), dopamine (DA), and/or acetylcholine, at a potency

comparable or even superior to that of conventional 5-HT and NE inhibitors and synthetic antidepressants; they concluded that **3** is the major if not only active constituent of St. John's Wort extracts,[19] acting by inhibiting neutrotransmitter metabolism.[20] However, the IC_{50} values presented in ref 19 for reuptake inhibition of the neurotransmitters by hyperforin compared to the entire extract clearly showed that **3** alone cannot be responsible for activity. The apparent inhibition of neurotransmitter uptake may be an artifact due to the interaction of the high concentration of the tested compounds with monoamine storage vesicles. Similarly, the inhibition of DA uptake by hyperforin and the structurally related adhyperforin was not due to a direct interaction with the typical binding site of antidepressants at the DA transporter, but rather to a noncompetitive interaction.[21] The apparent inhibition of serotonin uptake observed with the *H. perforatum* extract and **3** in vitro might also be due to an interaction of the compounds with Na^+ channels or Na^+/H^+ exchangers, leading to an increase in free intracellular sodium concentrations.[22,23] Such nonselective effects might explain why *H. perforatum* extracts and **3** block the synaptosomal uptake of multiple neurotransmitters.[19,24,25]

It is also noteworthy that the mechanisms underlying the complex disease of depression are still not well understood. On the other hand, even 15 years ago it was already known that neurotransmitter deficiency is not the only mechanism to lead to depression;[26] depression has also been linked to an inflammatory response since pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) are increased in depressed patients.[27−29] Pro-inflammatory cytokines interfere with the cellular immune system as well as neuroendocrine signaling pathways (for review, see ref 30), which lead to activation of the hypothalamic-pituitary-adrenal (HPA) axis. Elevated levels of CRH, ACTH, and cortisol have been observed in depressed patients,[31] which can be associated with a rise in pro-inflammatory cytokines.[32]

In our recent study we evaluated the mechanisms of action of a standardized *H. perforatum* extract in a rat chronic restraint stress model.[33] Markers of antioxidant capacity such as superoxide dismutase, glutathione peroxidase, and catalase in the hippocampus and hypothalamus and plasma levels of ACTH and corticosterone as well as the inflammatory markers IL-6 and TNF-α were determined in rats exposed to chronic restraint stress for 21 consecutive days. The *H. perforatum* extract and fluoxetine significantly reduced stress-induced increases in plasma ACTH and corticosterone levels. Furthermore, the administration of *H. perforatum* extract significantly reduced the stress-induced increase in TNF-alpha levels and normalized activities of antioxidant enzymes in the hippocampus and hypothalamus.[33] These data provide new evidence for the hypothesis that the mechanism of action of *H. perforatum* is mediated by the interrelationship between the immune, oxidative defense, and neuroendocrine system.

As mentioned previously, in vitro models relate to only one event and one aspect of the process (e.g., MAO inhibition, neurotransmitter reuptake inhibition). Since depression is a multifactorial and complex disease, animal models are frequently used in order to understand all aspects of the pathogenesis. Thus, we decided to take advantage of the well-validated forced swimming test (FST) in rats developed by Porsolt;[34] a significantly reduced time of the rat's immobility in comparison to a saline control indicates activity. The clear advantage of the FST compared to other animal models of depression is that it shows a good correlation to the clinical potency of synthetic antidepressants.[35] A bioguided fractionation of a commercially used *H. perforatum* extract (LI160) as well as oral application of fractions and isolates dosed relative to their content in the entire extract was used to detect the active constituents, in comparison to a standard antidepressant such as imipramine.

The crude *H. perforatum* extract showed significant activity in the FST at dosages between 125 and 1000 mg/kg rat.[36] Below and above, no activity was observed (so-called U-shaped activity)[37,38] (Table 1). Fractionation of the extract using Sephadex LH-20 yielded seven fractions, of which two were significantly active in the FST, whereas the others demonstrated minor activity. TLC and HPLC control of the active fractions showed for the more lipophilic fraction IIIc mainly hypericin (**1**) and pseudohypericin (**2**) as constituents. When testing pure **1**, it also exhibited activity in a U-shaped dose−response curve, but surprisingly at considerably higher amounts than present in the crude extract (Table 2).[39] Today the hypericins are widely accepted as the antidepressant constituents of St. John's Wort. In receptor-binding studies, Butterweck et al.[40] showed that hypericin (**1**) has high affinity for the D_3-dopamine receptor (K_i = 34.5 nM) and negligible affinities (K_i > 1000 nM) for nearly all other tested receptors and transporters. The affinity of **1** for the D_3-receptor subtype was much higher than that of the atypical antipsychotic clozapine (K_i = 372.3 nM). Others have shown that **1** has the most potent binding inhibition of all tested constituents to the human CRF_1 receptor (corticotrophin-releasing factor) with an IC_{50} value of 300 nM.[41] In a follow-up study, the same authors investigated the CRF-binding properties of hypericin (**1**) and pseudohypericin (**2**) in greater detail by measuring their effect on CRF-stimulated cAMP formation in recombinant Chinese hamster ovary (CHO) cells.[42] These authors found that only **1** strongly inhibited CRF binding with an IC_{50} of ca. 0.3 μM. In vivo, oral treatment of rats for two weeks with either *H. perforatum* extract or **1** significantly reduced plasma ACTH and corticosterone levels, suggesting a decrease in functional activity of the HPA axis.[43] In a more detailed study using an in situ hybridization technique, it was shown that hypericin (0.2 mg/kg) given daily by gavage for eight weeks significantly decreased levels of corticotropin-releasing hormone (CRH) mRNA by 16−22% in the hypothalamic paraventricular nucleus (PVN) and serotonin $5\text{-}HT_{1A}$ receptor mRNA by 11−17% in the hippocampus.[44] These results are of major interest since CRF1 receptor antagonists are under current

Table 1. Immobility Time in the Forced Swimming Test after Oral Administration of *H. perforatum* Extract[a]

treatment group	dose [mg/kg; po]	immobility [s] (mean ± SEM)
control		148.8 ± 9.2
imipramine	20	98.0 ± 15.0**
H. perforatum extract	60	127.9 ± 15.2
H. perforatum extract	125	92.5 ± 10.3**
H. perforatum extract	250	79.9 ± 15.6***
H. perforatum extract	500	88.6 ± 10.5***
H. perforatum extract	1000	87.3 ± 16.6**
H. perforatum extract	1500	135 ± 13.6

[a] $^{**}p < 0.01$; $^{***}p < 0.001$, $n = 9$.

Table 2. Immobility Time in the Forced Swimming Test after Oral Administration of Pure Hypericin (**1**) Alone and in Combination with Fraction $IIIc_1$, Which Was Given in a 10-fold Surplus[a]

treatment group	dose [mg/kg; po]	immobility [s] (mean ± SEM)
control		158.7 ± 8.9
imipramine	20	97.1 ± 10.2**
hypericin	0.08	154.7 ± 26.8
hypericin	0.23	110.2 ± 19.4**
hypericin	0.5	147.8 ± 20.6
fraction $IIIc_1$	10	133.9 ± 5.9
fraction $IIIc_1$	20	139.4 ± 21.9
hypericin + $IIIc_1$	0.001	127.3 ± 15.9
hypericin + $IIIc_1$	0.009	89.6 ± 18.5***
hypericin + $IIIc_1$	0.028	87.0 ± 21.6**
hypericin + $IIIc_1$	0.09	99.3 ± 15.4**
hypericin + $IIIc_1$	0.9	94.2 ± 14.0**
hypericin + $IIIc_1$	1.8	116.6 ± 29.6

[a] $^{**}p < 0.01$; $^{***}p < 0.001$, $n = 12-14$.

Table 3. Time of Immobility in the Forced Swimming Test after Oral Administration of Pure Hyperoside (**5**; quercetin 3-*O*-galactoside)[a]

treatment group	dose [mg/kg; po]	immobility [s] (mean ± SEM)
control		169.6 ± 19.0
imipramine	20	95.4 ± 8.4***
hyperoside	0.6	101.2 ± 11.3**
hyperoside	1.3	109.5 ± 12.1**
hyperoside	2.6	133.7 ± 9.3
hyperoside	5.2	149.2 ± 8.2
hyperoside	7.6	158.8 ± 7.6

[a] $^{**}p < 0.01$; $^{***}p < 0.001$, $n = 10$.

Table 4. Time of Immobility in the Forced Swimming Test after Oral Administration of the Flavonol Glycosides **6**, **7**, and **8** and the Flavanonol Glycoside Astilbin (**10**)[a]

treatment group	dose [mg/kg; po]	immobility [s] (mean ± SEM)
control		150.3 ± 11.1
imipramine	20	86.1 ± 7.4***
quercitrin (**6**)	0.6	149.9 ± 9.2
quercitrin (**6**)	1.3	162.3 ± 11.5
astilbin (**10**)	0.6	148.6 ± 8.6
astilbin (**10**)	1.3	163.7 ± 10.8
miquelianin (**8**)	0.6	89.4 ± 7.3***
isoquercitrin (**7**)	0.6	100.4 ± 9.7**

[a] $^{**}p < 0.01$; $^{***}p < 0.001$; $n = 10$.

development as potential pharmacotherapies for depression and anxiety disorders.[45]

The more hydrophilic active fraction II consisted of quercetin 3-*O*-glycosides with mainly hyperoside (**5**), quercitrin (**6**), and isoquercitrin (**7**). We tested pure **5**, **7**, miquelianin (**8**), quercitrin (**6**), and astilbin (**10**) at doses of 0.6 mg/kg rat (approximately the amount of hyperoside in the active dosage of the original fraction II). Of these, the first three compounds were active, whereas the latter two were not (Tables 3 and 4), indicating some specificity of activity probably due to the flavonol linked to the "physiologically adapted" hexoses such as glucose, galactose, and glucuronic acid.[46] At that time, almost 10 years ago, the finding that flavonol glycosides can exert antidepressant activity was hardly accepted, since if so, all vegetables and fruits should be able to treat depressive disorders. However, it seems that not every flavonoid glycoside is active and that flavonoid glycosides, when given in the form of enriched extracts without hydrolyzing enzymes (which are usually present in vegetables and fruits), are indeed therapeutically beneficial compounds. Meanwhile, flavonoids are well accepted as CNS-active compounds, which can reach the CNS even unchanged, as was shown for miquelianin (**8**) by in vitro experiments.[47]

In receptor-binding studies, quercetin (**4**) had an affinity to the D_4 receptor at $K_i = 7.8$ nM, miquelianin (**8**) to the α_{2C} receptor at 4 nM, and rutin (**9**) to the α_{2A} and α_{2c} receptors at 9 nM.[40] In the same study, amentoflavone (**14**) had a remarkable affinity for the δ-opioid receptor subtype in the nanomolar range ($K_i = 36.5$ nM) and significantly inhibited binding at the 5-HT_{1D}, 5-HT_{2C}, and dopamine D_3 receptors; however, this compound is present only in low concentrations in *H. perforatum* extracts, and tests with the much more abundant I3,II8-biapigenin (**13**) have not been performed (see above). It was shown in vivo[48] that hyperoside (**5**), isoquercitrin (**7**), and miquelianin (**8**) significantly down-regulate plasma ACTH and corticosterone levels after two weeks of daily treatment. The effect was similar to the synthetic antidepressant imipramine. Hypersecretion of ACTH and plasma cortisol have been reported in 40−50% of patients suffering from depression,[32,26] whereas normalization of the hyperactive HPA system occurs during successful antidepressant pharmacotherapy of depressive illnesses.[49] Flavonoids, therefore, could play an important role in the treatment of stress-related disorders due to their ability to decrease augmented HPA activity.

Newer results demonstrate that flavonoids are effective toward neurodegeneration and may be used as modulators of cellular signal transduction in the MAPK signaling pathway.[50] They also may exert beneficial effects in the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation, and by promoting neurocognitive performance.[51] These data clearly prove that flavonoids not only could play an important role in the treatment of depressive illnesses but also contribute to the beneficial effects of St. John's Wort extract after oral application.

At this point we can draw an **initial conclusion** from the first lesson learned from herbals: A therapeutically effective herbal extract does not contain just one active constituent or a few structurally similar compounds, but several often structurally unrelated groups of compounds may occur with activity that influences the same pathological situation. In the case of *H. perforatum*, these are the hypericins, some flavonol glycosides, and the hyperforins; it is obvious that their mechanism of action is different (see above), but especially suitable for complex, multiple-caused diseases such as depression. A similar (multifunctional) situation is true for other well-known herbal drugs, e.g., chamomile for inflammation, licorice for bowel disorders, willow bark as an analgesic, ginkgo leaf for increased brain blood flow, and garlic as an antilipidemic, just to name a few for which the phytochemistry and pharmacology have been comparatively well investigated.

Pharmacokinetic Synergy of Constituents in *H. perforatum*. It was mentioned above that the amount of pure hypericin (**1**) to exert significant antidepressant activity in the FST was exceptionally high (0.23 mg/kg, po), whereas fraction IIIc, which was rich in hypericin, was active at doses of 0.028−0.166 mg/kg (po). Assuming that there were additional active compounds, we investigated this fraction further phytochemically; subfraction IIIc1 was obtained containing some procyanidins, in particular, procyanidin B2 (**11**). However, this subfraction did not show any activity in the FST, but when pure hypericin (**1**) was combined with subfraction IIIc1, significant activity was observed in the FST, in doses ranging from 0.009 to 0.9 mg hypericin/kg (Table 2).[39]

The question was raised, by which mechanism do the nonactive procyanidins increase the pharmacological activity of hypericin? Solubility experiments of hypericin (**1**) in water in the presence and absence of pure dimeric procyandin B2 (**11**) and trimeric procyanidin C1 (**12**) provided the solution to this question. **1** alone was poorly soluble in water at approximately 0.5 μg/mL. With increasing amounts of **11**, the solubility of hypericin (**1**) was increased to more than 60 μg/mL (hypericin:procyanidin B2 was 1:25), and with procyanidin C1 (**12**) to 40 μg/mL (ratio 1:25). Interestingly, the monomeric components of the oligomeric procyanidins, catechin and epicatechin, did not increase the solubility of hypericin.[39] We further demonstrated that other polyphenols present in *Hypericum* extracts may also influence the solubility of **1** in water. In a further investigation we used the octanol/buffer (pH 7.4) partition coefficient (log *P*) of **1** as determined by HPLC in octanol as well as the aqueous phase (Figure 1).[52] Without any so-called co-effector, log *P* of **1** was approximately 4.8, indicating that almost all the hypericin was present in the octanol phase; with the most active co-effector miquelianin (**8**) it was approximately 2.5, showing that the amount of **1** in the water phase was increased by about 180-fold. Other phenolic compounds present in *H. perforatum* possessed less solubilization activity, as shown for procyanidin B2 (**11**; 76-fold) to amentoflavone (**14**; 4-fold) (Figure 1).

Does the increased solubility of hypericin (**1**) lead to increased in vivo activity? When feeding pure **1** orally to rats, the plasma concentration of hypericin increased steadily to about 6 ng/mL at 270 min and then slowly decreased (the AUC was set at 100%). When giving **1** with the co-effector procyanidin B2 (**11**; 0.2/2.5

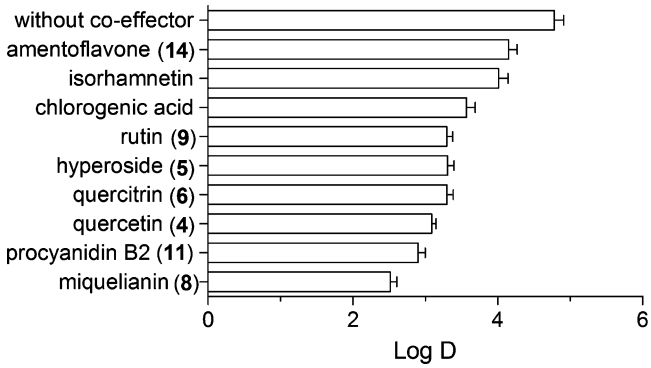


Figure 1. Octanol/buffer (pH 7.4) coefficient of hypericin (**1**) before (without co-effector) and after addition of phenolic substances (co-effectors) present in *H. perforatum* (proportion hypericin + co-effector x = 1 + 5).

mg/kg), the AUC was increased to 160% with indications for a biphasic absorption. In the presence of hyperoside (quercetin 3-*O*-galactoside; **5**) a similar biphasic absorption was observed with an increase to 140%.[53] Thus, the increased solubility of **1** in the presence of a proanthocyanidin or a quercetin glycoside indeed increases its bioavailoability in rats. It can be assumed that this can be transferred to the therapeutic situation in patients.

Meanwhile, papers have been published[54] (for review, see ref 55) that indicate an influence of compounds present in *H. perforatum* on transport systems of the small intestine cells. Usually such transport studies are performed with the aim to detect metabolic interactions that lead to increased or reduced plasma levels of important medications. It was our approach to use the well-established Caco-2 cell model to investigate phenolic compounds of *H. perforatum* for their ability to increase the transport and thus the bioavailability of hypericin (**1**) through the intestinal cells. This monolayer model of colon adenocarcinoma cells in a Transwell chamber shows good qualitative similarity to the function of the cells of the small intestine[56] and provides the possibility of measuring the transport from the apical to the basolateral site (reflecting the absorption of a given compound to the plasma site) and from the basolateral to the apical site (reflecting the backward transport, the efflux from the small intestine cell to the lumen).[52] When dosing pure **1** to the apical site, there was only a very low transport to the basolateral site, indicating a poor transport of this compound (about 0.25×10^{-6} cm/s). When dosing hypericin to the basolateral site, we measured a strong apical-directed transport of more than 1.2×10^{-5} cm/s, which explains the poor transport to the basolateral site. Phloridzin (an inhibitor of the sodium-dependent glucose transporter 1) and the synthetic MK-571 (which inhibits the MRP-1/2 transporter) reduced the efflux to about 3×10^{-6} cm/s, thereby increasing the transport value to the basolateral site to about 2×10^{-6} cm/s. Verapamil, an inhibitor of P-glycoprotein (an efflux transporter of many xenobiotics), reduces the apical and the basolateral transport only marginally (Figure 2). A series of constituents of *H. perforatum* (in the order astilbin (**10**), rutin (**9**), hyperoside (**5**), biapigenin (**13**), procyanidin B2 (**11**), miquelianin (**8**), isoquercitrin (**7**)) decrease the efflux from more than 1×10^{-5} to 3.5×10^{-6} and increase the basolateral transport of **1** from below 1×10^{-6} to almost 2×10^{-6} cm/s (Figure 2). Although these data were obtained by in vitro experiments, it is likely that several polyphenols in *H. perforatum* play a role as co-effectors in that they decrease its apical (backward) transport and thus increase the concentration of hypericin at the plasma site in patients.

The lesson that we have learned from these data can be formulated as a **second conclusion**: the bioavailability of certain active constituents in an herbal, in the case of *H. perforatum* hypericin (**1**), can be improved by accompanying compounds (so-called co-effectors) present in the same botanical product. The

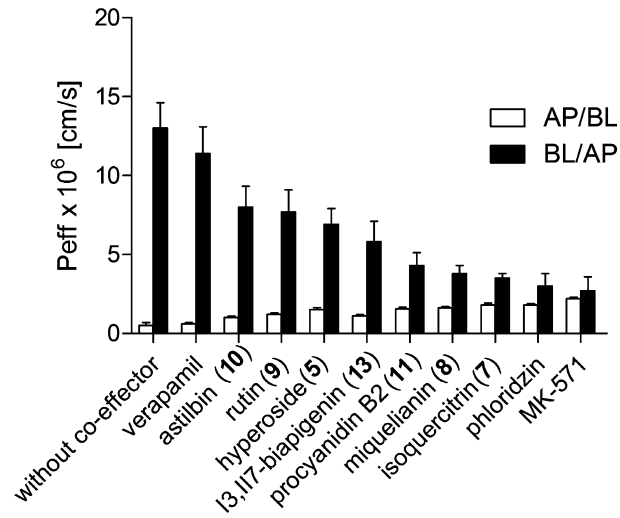


Figure 2. Permeation coefficient of hypericin (**1**; 10 μg/mL) in Caco-2 cells without co-effector, together with the control compounds verapamil (49 μg/mL), phloridzin (226 μg/mL), and MK 571 (27 μg/mL) and in the presence of phenolic co-effectors from *H. perforatum* (all 100 μg/mL). White: apical (AP) to basolateral (BL). Black: BL to AP.

pharmacokinetic mechanisms can be physicochemical, i.e., enhanced solubility, or biochemical, i.e., increased transport to the plasma site. An aspect that was not investigated here, but that may also occur in some cases, is decreased phase I/II metabolism. These types of interactions between compounds are combined under the term "pharmacokinetic synergy"; unfortunately, they are not well investigated in herbals, particularly at the molecular level. The observation has some common consequences for research with botanicals: (i) Bioguided fractionation of an active extract could result in a loss of activity of potentially active fractions or single compounds since co-effectors may be present in the crude plant material for which the concentration can be decreased during the purification process. (ii) The concept of co-effectors can be also applied to toxicity data: when an active plant extract is less toxic than its fractions or isolates. In this case, solubilization of a toxic compound may be decreased; its efflux transport as well as phase I/II enzymes may be activated (this concept seems to be widely used empirically in TCM). (iii) Pharmacokinetic synergy also points to the often observed situation that plant extracts can be dosed to lower amounts relative to their isolates, an effect that has misleadingly resulted in the statement that herbal medicinal products cannot work therapeutically because of their low dosage (so-called "pseudoplacebos"[57]).

Unwanted Side Effects of *H. perforatum*. Clinical observations in the late 1990s, when commercial extracts with high hyperforin (**3**) content were developed and made available on the market, have led to the finding that, in vivo, *H. perforatum* extracts activated the phase I enzyme system CYP3A4,[58−60] causing a decrease in plasma concentration of important drugs such as digoxin, tacrolimus, or cyclosporine. When the pure constituents hypericin (**1**), hyperforin (**3**), and I3,II8-biapigenin (**13**) were tested in vitro, they were detected as inhibitors of CYP3A4, with IC_{50} values from 8.7, 2.3, and 0.08 μM, respectively.[61] Incidentally, this is an excellent example that in vitro findings cannot necessarily be transferred to the in vivo situation. These inconsistent data were clarified by the findings of Moore et al.,[62] who used human hepatocytes to show that **3** is a potent ligand (K_i = 27 nM) for the pregnane X receptor, an orphan nuclear receptor that regulates and activates the expression of the cytochrome P450 (CYP) 3A4 monooxygenase. *H. perforatum* extracts and rifampicin as a positive control increased the monooxygenase activity about 6- to 7-fold at 10 μg/mL, whereas pure hypericin (**1**), amentoflavone (**14**), and

Table 5. Activity in the Forced Swimming Test of Extracts A, B, and C (see text) after Chronic Application (14 days, once a day)[a]

treatment group	dose [mg/kg; po]	immobility [s] (mean ± SEM)
control		138.6 ± 12
imipramine	20	77.5 ± 11**
extract A	250	145 ± 8
extract A	500	97 ± 14*
extract B	250	118 ± 19
extract B	500	97 ± 14*
extract C	250	110 ± 11
extract C	500	93 ± 8*

[a] *$p < 0.05$; **$p < 0.01$.

several flavonol glycosides and aglycones increased it only 1- to 2-fold at the same concentration. Hyperforin (**3**), in contrast, increased the enzyme activity by more than 6-fold when used at a concentration of 1 μM; however, concentrations > 1 μM proved to be toxic. These findings provided the first evidence that **3** could be the constituent responsible for the clinically observed interactions. Later on, clinical studies showed that a commercial extract of *H. perforatum* with a high concentration of hyperforin (4.66%) significantly reduced the plasma concentration of cyclosporin, whereas the same extract almost free of **3** (0.33%) did not.[63]

In view of these data, the questions arise as to whether hyperforin (**3**) is indeed the major active compound of *H. perforatum* extracts, as postulated earlier,[20,64] and if extracts low in or free of hyperforin still would possess antidepressant activity. In order to clarify these questions, we used a crude extract A with quantified amounts of hyperforin (32 mg/g), hypericins (1.5 mg/g), and flavonoids (84 mg/g) and removed hyperforin using supercritical CO_2, resulting in extract B with hypericins (1.4 mg/g) and flavonoids (82 mg/g) and further removed the hypericins from B to yield extract C with flavonoids only (104 mg/g). All extracts (A, B, C) exhibited significant activity in the chronic FST at a dosage of 500 mg/kg rat (Table 5).[65] Thus, not only without hyperforin but also with only flavonoids present, *H. perforatum* extracts are pharmacologically active in the FST. Our results supported a clinical study by which a commercial extract (ZE 117; <0.4% **3**), low in hyperforin, was superior to placebo, but similarly efficient in comparison to imipramine and fluoxetine.[66,67] It can be concluded that hyperforin (**3**) is not that essential for the antidepressant activity as originally assumed;[19] the results however point to the group of flavonoids, which may be more important for antidepressant activity than hitherto believed.

These results finally lead to the **third conclusion**: During the manufacturing process it is possible to eliminate or degrade even unwanted active constituents from a herbal medicine in order to produce safe botanical extracts with retained therapeutic efficacy. Elimination of unwanted constituents/fractions may also lead to so-called "refined extracts", which in some cases may be patentable.

Conclusion

Comprehensive research on the active constituents of traditionally used herbals, their isolation by bioguided fractionation, and their identification is necessary to get insight into their chemistry, biological activity (pharmacology *and* toxicology), and synergistic properties. These data allow optimization of the pharmaceutical and thereby the therapeutic quality of any particular herbal medicinal product. The current quality requirements for the internal use of *H. perforatum* extracts for the treatment of depression apply to the hypericins, which should be in the extract at a concentration of 0.1 to 0.3%, the flavonol glycosides at 6 to 12%, and hyperforin (**3**) *below* 2%. The flavonoids additionally play an important role as co-effectors for improving the biopharmaceutical properties of hypericin (**1**); the same can be applied for the procyanidins, which should be in the extract in a concentration of not less than 10%. Some of these requirements already have been realized in the European Pharmacopoeia.[68] The monograph indicates that hyperforin (**3**) content can be kept low if the plant is collected before major development of the fruits (definition in the monograph: "the flowering upper parts") since these besides the flowers are particularly rich in hyperforin (for reference see ref 5). Stabilization of **3** with antioxidants added to the extract should therefore not be allowed. The required drug:extract ratio (DER) of 4−7:1 can be achieved with 80% methanol or 60% ethanol for extraction and should be taken seriously because it cannot be ruled out that hitherto undetected active constituents and co-effectors are among the 30−40% of unknown compounds. Thus, the entire extract still has to be considered as the active constituent of St. John's wort, to elicit its antidepressant effects.

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References and Notes

(1) Anonymous. *World Health Report 2001: Mental Health, New Understanding, New Hope*; WHO: Geneva, 2001.
(2) Linde, K.; Ramirez, G.; Mulrow, C. *Br. Med. J.* **1996**, *313*, 253–258.
(3) Clement, K. C.; Johnson, M. J.; Dearing, K. *Holist. Nurs. Pract.* **2006**, *20*, 197–203.
(4) Bombardelli, E.; Morazzoni, P. *Fitoterapia* **1995**, *66*, 43–68.
(5) Nahrstedt, A.; Butterweck, V. *Pharmacopsychiatry* **1997**, *30* (Suppl. 2), 129–134.
(6) Nahrstedt, A. In *Herbal Medicinal Products for the Treatment of Pain*; Chrubasik, S.; Roufogalis, B. D., Eds.; Southern Cross University Press: Lismore, Australia, 2000; pp 144−153.
(7) Falk, H.; Schmitzberger, W. *Monatsh. Chem.* **1992**, *123*, 731–739.
(8) Ang, C. Y.; Hu, L.; Heinze, T. M.; Cui, Y.; Freeman, J. P.; Kozak, K.; Luo, W.; Liu, F. F.; Mattia, A.; DiNovi, M. *J. Agric. Food Chem.* **2004**, *52*, 6156–6164.
(9) Bystrov, N. S.; Chernov, B. K.; Dobrynin, V. N.; Kolosov, M. N. *Tetrahedron Lett.* **1975**, 2791–2794.
(10) Verotta, L.; Appendino, G.; Jakupovic, J.; Bombardelli, E. *J. Nat. Prod.* **2000**, *63*, 412–415.
(11) Chialva, F.; Gabri, G.; Liddle, P. A. P.; Ulian, F. *J. High Res. Chromatog. Chromatogr. Commun.* **1982**, *5*, 182–188.
(12) Jürgenliemk, G.; Nahrstedt, A. *Planta Med.* **2002**, *68*, 88–91.
(13) Sparenberg, B. L.; Demisch, J.; Hölzl, J. *Pharm. Ztg. Wiss.* **1993**, *138*, 239–254.
(14) Ploss, O.; Petereit, F.; Nahrstedt, A. *Pharmazie* **2001**, *56*, 509–511.
(15) von Eggelkraut-Gottanka, S. G.; Abu Abed, S.; Muller, W.; Schmidt, P. C. *Phytochem. Anal.* **2002**, *13*, 170–176.
(16) Suzuki, O.; Katsumata, Y.; Chari, M.; Vermes, B.; Wagner, H.; Hostettmann, K. *Planta Med.* **1981**, *42*, 17–21.
(17) Bladt, S.; Wagner, H. *J. Geriatr. Psychiatry Neurol.* **1994**, *7* (Suppl. 1), S57–59.
(18) Baureithel, K. H.; Büter, K. B.; Engesser, A.; Burkard, W.; Schaffner, W. *Pharm. Acta Helv.* **1997**, *72*, 153–157.
(19) Chatterjee, S.; Bhattacharya, S.; Wonnemann, M.; Singer, A.; Müller, W. *Life Sci.* **1998**, *63*, 499–510.
(20) Chatterjee, S. S.; Nöldner, M.; Koch, E.; Erdelmeier, C. *Pharmacopsychiatry* **1998**, *31* (Suppl. 1), 7–15.
(21) Jensen, A. G.; Hansen, S. H.; Nielsen, E. O. *Life Sci.* **2001**, *14*, 1593–1605.
(22) Wonnemann, M.; Singer, A.; Müller, W. E. *Neuropsychopharmacology* **2000**, *23*, 188–197.
(23) Wonnemann, M.; Singer, A.; Siebert, B.; Müller, W. E. *Pharmacopsychiatry* **2001**, *41* (Suppl. 1), S148–S151.
(24) Gobbi, M.; Dalla Valle, F.; Ciapparelli, C.; Diomede, L.; Morazzoni, L. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *360*, 262–269.
(25) Singer, A.; Wonnemann, M.; Müller, W. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 1363–1368.
(26) Holsboer, F.; Barden, N. *Endocr. Rev.* **1996**, *17*, 187–205.
(27) Thiele, B.; Brink, I.; Ploch, M. *J. Geriatr. Psychiatry Neurol.* **1994**, *7*, S60–S62.
(28) Anisman, H.; Hayley, S.; Turrin, N.; Merali, Z. *Int. J. Neuropsychopharmacol.* **2002**, *5*, 357–373.
(29) Miller, A. H.; Maletic, V.; Raison, C. L. *Biol. Psychiatry* **2009**, *65*, 732–741.
(30) Schiepers, O. J.; Wichers, M. C.; Maes, M. *Progr. Neuropsychopharmacol. Biol. Psychiatry* **2005**, *29*, 201–217.
(31) Plotsky, P. M.; Owens, M. J.; Nemeroff, C. B. *Psychiatr. Clin. North Am.* **1998**, *21*, 293–307.

(32) Gold, P. W.; Licinio, J.; Wong, M. L.; Chrousos, G. P. *Ann. N.Y. Acad. Sci.* **1995**, *771*, 716–729.
(33) Grundmann, O.; Lv, Y.; Kelber, O.; Butterweck, V. *Neuropharmacology* **2010**, *58*, 767–773.
(34) Porsolt, R.; Le Pichon, M.; Jalfre, M. *Nature* **1977**, *266*, 730–732.
(35) Porsolt, R. D. In *Antidepressants: Neurochemical, Behavioural and Clinical Perspectives*; Enna, S. J.; Malick, J. B.; Richelson, E., Eds.; Raven Press: New York, 1981; pp 121−139.
(36) Butterweck, V.; Wall, A.; Liefländer-Wulf, U.; Winterhoff, H.; Nahrstedt, A. *Pharmacopsychiatry* **1997**, *30* (Suppl.), 117–124.
(37) Calabrese, E. J.; Baldwin, L. A. *Ann. Rev. Public Health* **2001**, *22*, 15–33.
(38) Calabrese, E. J. *Crit. Rev. Toxicol.* **2008**, *38*, 591–598.
(39) Butterweck, V.; Petereit, F.; Winterhoff, H.; Nahrstedt, A. *Planta Med.* **1998**, *64*, 291–294.
(40) Butterweck, V.; Nahrstedt, A.; Evans, J.; Rauser, L.; Savage, J.; Popadak, B.; Ernsberger, P.; Roth, B. L. *Psychopharmacology* **2002**, *162*, 193–202.
(41) Simmen, U.; Burkard, W.; Berger, K.; Schaffner, W.; Lundstrom, K. *J. Recept. Signal Transduct. Res.* **1999**, *19*, 59–74.
(42) Simmen, U.; Higelin, J.; Berger-Büter, K.; Schaffner, W.; Lundstrom, K. *Pharmacopsychiatry* **2001**, *34* (Suppl. 1), S137–S142.
(43) Butterweck, V.; Korte, B.; Winterhoff, H. *Pharmacopsychiatry* **2001**, *34* (Suppl. 1), S2–S7.
(44) Butterweck, V.; Winterhoff, H.; Herkenham, M. *Mol. Psychiatry* **2001**, *6*, 547–564.
(45) Vandenbogaerde, A.; Zanoli, P.; Puia, G.; Truzzi, C.; Kamuhabwa, A.; De Witte, P.; Merlevede, W.; Baraldi, M. *Pharmacol., Biochem. Behav.* **2000**, *65*, 627–633.
(46) Butterweck, V.; Jürgenliemk, G.; Nahrstedt, A.; Winterhoff, H. *Planta Med.* **2000**, *66*, 3–6.
(47) Jürgenliemk, G.; Boje, K.; Hüwel, S.; Lohmann, C.; Galla, H. J.; Nahrstedt, A. *Planta Med.* **2003**, *69*, 1013–1017.
(48) Butterweck, V.; Hegger, M.; Winterhoff, H. *Planta Med.* **2004**, *70*, 1008–1011.
(49) Barden, N.; Reul, J. M.; Holsboer, F. *Trends Neurosci.* **1995**, *18*, 6–11.
(50) Hagen Schroeter, C. B.; Jeremy, P. E.; Spencer, R. J. W.; Enrique Cadenas, C. R.-E. *Neurobiol. Aging* **2002**, *23*, 861–880.
(51) Spencer, J. P. E. *Genes Nutr.* **2007**, *2*, 257–273.
(52) Sieger, R.; Nahrstedt, A. *Planta Med.* **2006**, *72*, 1073.
(53) Butterweck, V.; Liefländer-Wulf, U.; Winterhoff, H.; Nahrstedt, A. *Planta Med.* **2003**, *69*, 189–192.
(54) Xie, H. G. *Clin. Pharmacol. Ther.* **2005**, *78*, 440–441.
(55) Nowack, R. *Nephrology* **2008**, *13*, 337–347.
(56) Artursson, P.; Karlsson, J. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880–885.
(57) Wiesing, U. *Wer heilt, hat Recht: Über Pragmatik und Pluralität in der Medizin.*: Schattauer Verlag: Stuttgart, 2004.
(58) Mannel, M. *Drug Safety* **2004**, *27*, 773–797.
(59) Zhou, S.; Chan, E.; Pan, S. Q.; Huang, M.; Lee, E. J. *J. Psychopharmacol.* **2004**, *18*, 262–276.
(60) Madabushi, R.; Frank, B.; Drewelow, B.; Derendorf, H.; Butterweck, V. *Eur. J. Clin. Pharmacol.* **2006**, *62*, 225–233.
(61) Unger, M. *Dtsch. Apoth. Ztg.* **2004**, *149*, 979–986.
(62) Moore, L. B.; Goodwin, B.; Jones, S. A.; Wisely, G. B.; Serabjit-Singh, C. J.; Willson, T. M.; Collins, J. L.; Kliewer, S. A. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 7500–7502.
(63) Mai, I.; Bauer, S.; Perloff, E. S.; Johne, A.; Uehleke, B.; Frank, B.; Budde, K.; Roots, I. *Clin. Pharmacol. Ther.* **2004**, *76*, 330–340.
(64) Müller, W. E. *Pharmacol. Res.* **2003**, *47*, 101–109.
(65) Butterweck, V.; Christoffel, V.; Nahrstedt, A.; Petereit, F.; Spengler, B.; Winterhoff, H. *Life Sci.* **2003**, *73*, 627–639.
(66) Woelk, H. *Br. Med. J.* **2000**, *15*, 4.
(67) Kaeufeler, R.; Meier, B.; Brattström, A. *Pharmacopsychiatry* **2001**, *34* (Suppl. 1), S49–S50.
(68) Anonymous. In *Europäisches Arzneibuch*; Deutscher Apothekerverlag: Stuttgart, 2009; Vol. 6, Suppl. 3, pp 5515−5516.

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