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Dissolution, solubility and cooperativity of phenolic compounds from *Hypericum perforatum* L. in aqueous systems

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Received August 14, 2002, accepted October 21, 2002

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Pharmazie 58: 200–203 (2003)

The dissolution in water of phenolic constituents of St. John's wort (*Hypericum perforatum* L.) from a medicinal tea and a coated tablet formulation showed different dissolution profiles. In general, the flavonoid glycosides were well dissolved, followed by flavonoid aglycones and hypericin while hyperforin was only detectable at a very low level. Interestingly, hypericin exhibited much better extraction and dissolution rates than the similarly lipophilic hyperforin. When determining the octanol/water partition coefficient it became obvious that the solubility of pure hypericin in water increased upon addition of some phenolic constituents typical for *Hypericum* extracts. Most effective in solubilizing hypericin was hyperoside (hyperin, quercetin 3-*O*-β-D-galactoside) which increased the concentration of hypericin in the water phase up to 400fold in this model.

1. Introduction

Extracts of the flowering upper parts of St. John's wort (*Hypericum perforatum* L.) are used in the treatment of mild and moderate depression [1]. Modern application forms such as dragees, tablets and capsules are frequently used but still the traditional water infusion from the herbal tea is prepared for therapeutical purposes. Although the antidepressant activity of the plant is shown in many clinical studies and *in vivo* and *in vitro* pharmacological models, the active constituents of *Hypericum* are not fully known [2]: phloroglucinols (hyperforin) [3], naphthodianthrones (e.g. hypericin) [4] and flavonoid monoglycosides (hyperoside, isoquercitrin, miquelianin) [5] are discussed as antidepressant active constituents. These compounds show wide differences in their hydrophilic/lipophilic properties indicating differences in their solubility in aqueous systems. Because solubility is important for the pharmacological effectiveness of a drug [6], the aim of this study was to investigate exemplarily the extraction rates of main phenolic constituents from a herbal tea and the dissolution properties of a coated tablet. It has been shown earlier that proanthocyanidins, such as procyanidin B2, increase the solubility of hypericin in aqueous systems [4]; to obtain more insight into such cooperative effects we tested additional main phenolic constituents of *Hypericum* for their ability to increase water solubility of one of the active compounds, hypericin.

2. Investigations, results and discussion

For 100% values the various products were extracted with 80% MeOH or MeOH/DMSO mixtures followed by quantitative HPLC [7]. Infusions of the herbal tea were prepared with boiling water and 100 mg of the dry residue were dissolved in MeOH and taken for HPLC measurement; dissolution tests were performed as described in the European Pharmacopoeia [8] using a paddle apparatus with 500 ml H_2O; 5 ml samples were diluted with MeOH and quantified by HPLC [7].

When preparing an infusion, the extraction rate depends on a number of different factors such as homogeneity of the material, particle seize, water temperature [9] which often vary in batch and thus in one series of experiments. This results in a comparatively large variation as shown in Fig. 1, even when the values were calculated from four different infusions. As expected for the aqueous phase, the yield of phenolic constituents decreased with increasing lipophilicity of the compounds. 60 to 80% of the more polar phenolic compounds such as quinic acid derivatives and flavonoid glycosides, mainly quercetin glycosides, were present in the infusion, whereas the extraction rates of non-glycosylated flavonoids (quercetin), naphthodianthrones and the phloroglucinol hyperforin were much less. In spite of the comparable lipophilicity of the hypericins and hyperforin (when estimated by their comparable chromatographic behaviour on RP phases), hypericin (c. 12%) and pseudohypericin (c. 22%) were clearly better extracted than hyperforin (c. 1%) with values that corroborate earlier data in the literature for hypericin and pseudohypericin [10].

In contrast to a simple herbal infusion, the dissolution of a solid dosage formulation is a complex event since it is far more difficult to predict the dissolution of its constituents. A coated tablet was chosen as an example of a solid application form. The dissolution test was performed in triplicate in water. Fig. 2 shows that again phenolic glycosides are better dissolved (90 to 100%; 60 min) than flavonoid aglycones (35 to 40%; 60 min) followed by hypericin (40%; 60 min) and hyperforin (2%; 60 min). As already indicated by the first investigations, the dissolution

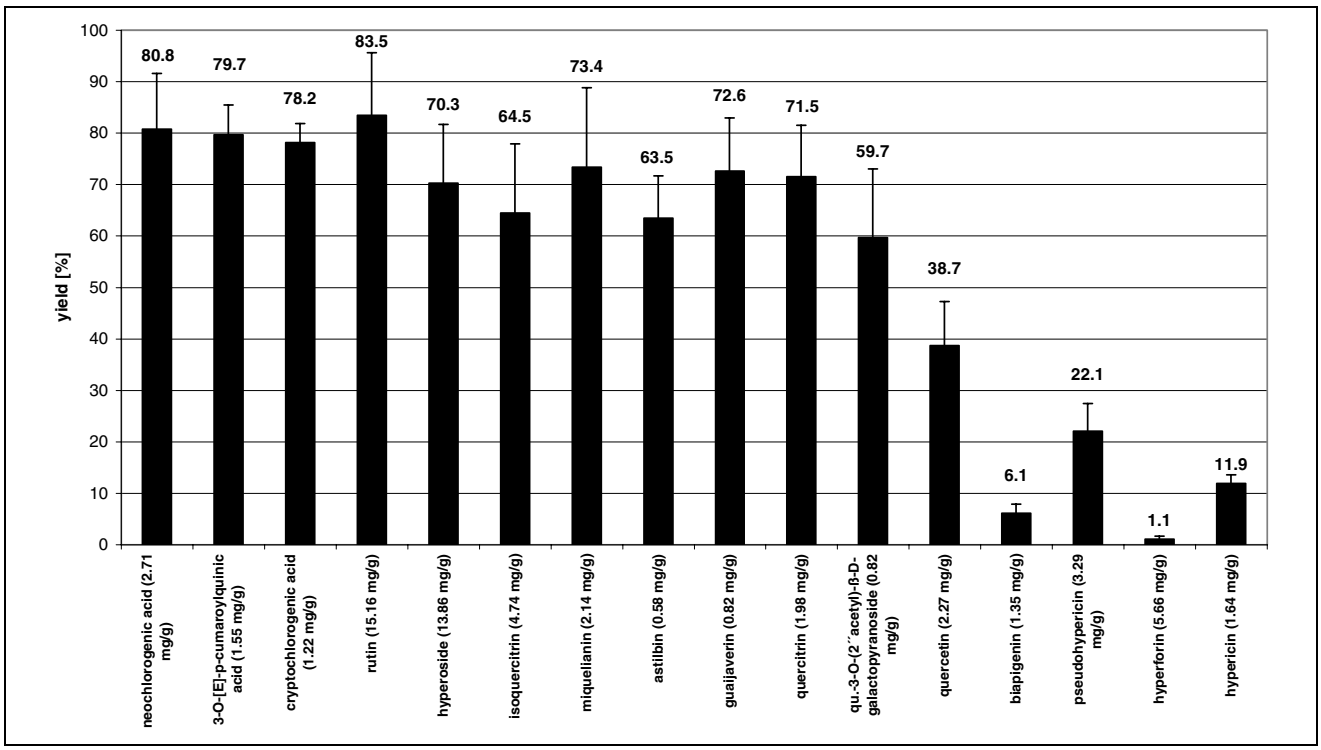

Fig. 1: Yields of phenolic constituents in an infusion prepared from St. John's wort in percent of the amount present in the crude drug (n = 4); 100 % values are presented in brackets after each constituent measured

of hypericin in water was clearly better than that of the similar lipophilic hyperforin.

The dissolution behaviour of hypericin in the above tests that appeared irregularly in view of its well known bad solubility in water as a pure compound was further investigated. Butterweck et al. showed an increased solubility of hypericin in aqueous solution when a procyanidin fraction isolated from *H. perforatum* and in particular pure procyanidin B2, was added [4]. In order to investigate a similar coeffective behaviour of additional phenolic compounds from *H. perforatum*, the octanol/water partition coefficient (P_{Oct}) was used. After partition of hypericin in the absence or presence of different amounts of the main flavonoid glycosides of *H. perforatum* between water and octanol, the hypericin concentration was determined in the water phase (Fig. 3): While the aglycone quercetin in-

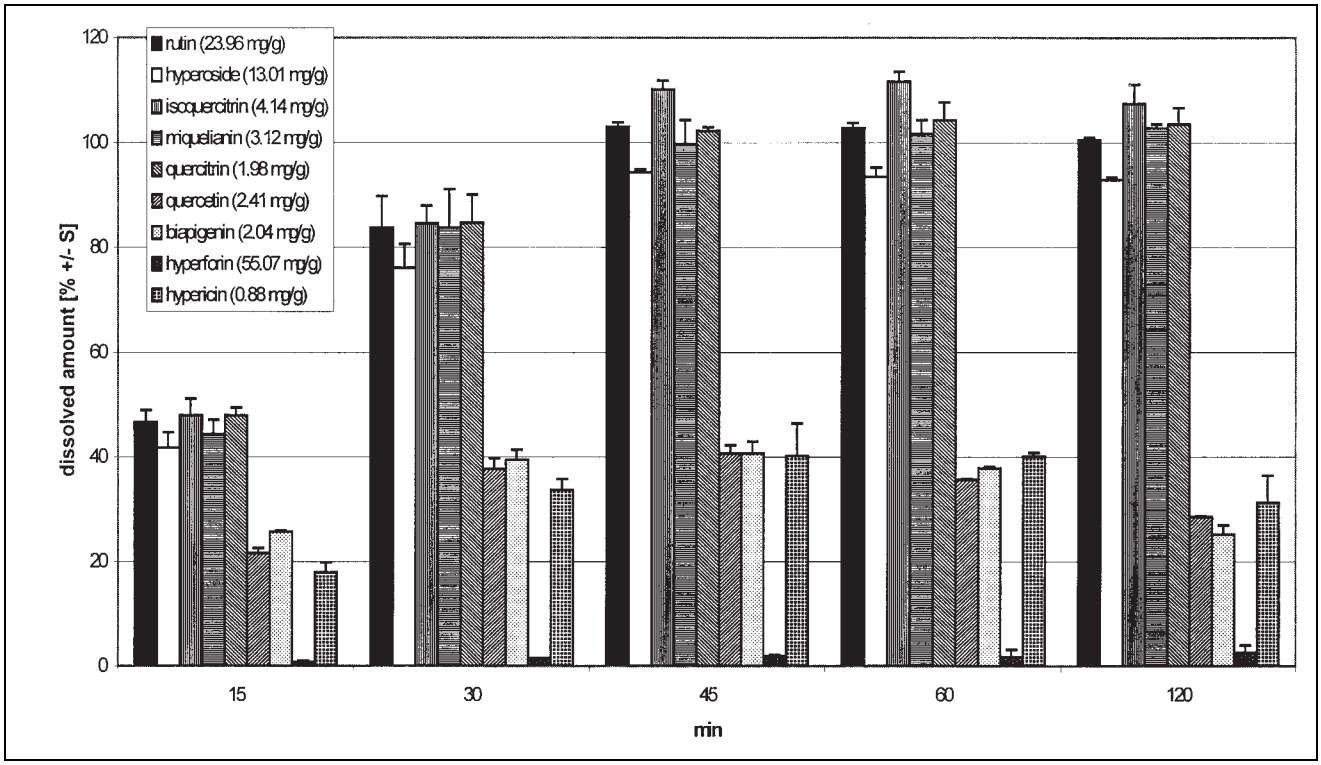

Fig. 2: Yields of phenolic constituents in the dissolution test of a coated tablet in percent of the amount present in the tablet (n = 3); 100% values are presented in brackets after each constituent measured

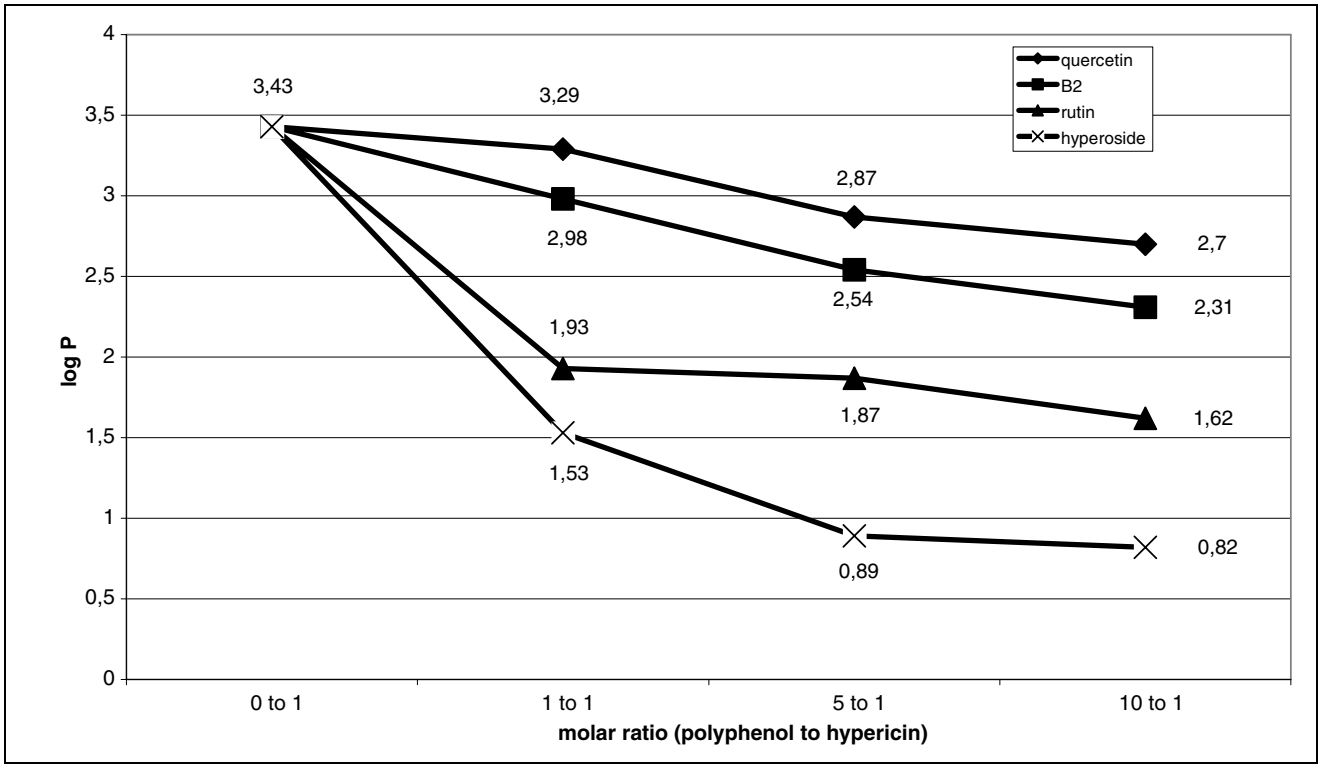

Fig. 3: Octanol/water-partition coefficient of 1 mg hypericin in the presence of vaious amounts of *Hypericum* polyphenols

creased the concentration of hypericin fivefold in the water phase (P_{Oct} of hypericin = 2692, log P = 3.43; P_{Oct} of hypericin with quercetin 1 : 10 = 501, log P = 2.7), the procyanidin B2 was more effective in the same molar ratio (P_{Oct} of hypericin in the presence of B2 1 : 10 = 204, log P = 2.3). However, the quercetin glycosides rutin (quercetin 3-*O*-rutinoside) and hyperoside (quercetin 3-*O*-β-D-galactoside) increased the solubility of hypericin already at a 1 : 1 molar ratio more effectively than the aglycones at a 10 : 1 ratio. Furthermore, at a tenfold molar surplus of hyperoside, hypericin showed a P_{Oct} of 7 (log P = 0.82) pointing to a c. 400 fold higher concentration of hypericin in the water phase than without hyperoside; the corresponding value for rutin was $P_{Oct} = 42$ (log P = 1.62).

These data show that in addition to their own pharmacological activity as antidepressants in the Forced Swimming Test [5] the flavonoids increase the generally bad dissolution of hypericin, another active compound in the same *in vivo* test [4]. The data explain the observed relatively good solubility of hypericin in water from the crude drug or formulations of *H. perforatum* in contrast to pure hypericin. Though already indicated indirectly [4], further investigations will show whether the presence of flavonoids such as rutin and hyperoside, and the procyanidins such as B2 indeed cause a better bioavailability of hypericin than without these cooperative compounds. Nevertheless, this example nicely shows the advantage of complex extracts over pure compounds in terms of biopharmaceutical properties [6], that is often discussed [11] but rarely intensively studied.

3. Experimental

3.1. Materials

Coated tablet: Neuroplant® 300 (Spitzner/Schwabe, Karlsruhe (Germany), Ch-B.: 0540599); medicinal tea: Sidroga® Johanniskraut (Sidroga GmbH, Bad Säckingen (Germany), Ch-B.: 000404 A0830052); Octanol-(1) and other chemicals from Merck, Darmstadt (Germany); hypericin, rutin and hyperoside from Roth KG, Karlsruhe (Germany); procyanidin B2 was isolated in this Institute, its purity was checked by HPLC and NMR; dissolution-apparatus: Pharma Test paddle apparatus, Type PTW, No. 8-124/2, Hainburg (Germany)

3.2. Quantification of a tea extract

Two teabags (3.5 g drug) were poured over with 150 ml seething-hot tap water. After 5 min the bags were moved 5 times up and down. After 10 min the bags were squeezed out and the solution was dried by lyophilization. 100 mg of the extract were quantified by HPLC [7]. The work was performed prevented from direct light.

For reference data 12.3 g of the teabag's content were totally extracted with 80% MeOH using an ultra-turrax and analysed by HPLC [7].

3.3. Dissolution test

The dissolution tests were performed according to the European Pharmacopoeia [8] using a paddle apparatus with 500 ml medium (tap water of pH 7.3) at 37 °C and a paddle speed of 150 rpm. At 15, 30, 45, 60 and 120 min 5 ml samples were taken and medium of 37 °C was replenished. The samples were centrifuged 10 min at 3000 rpm. 5 µl of the internal standard solution (7-*O*-methyl-pinocembrin) were added to 4 ml of the clear solution and MeOH added to 10.0 ml. The sample was quantified by HPLC as described in [7]. Procedures were prevented from direct light. All experiments were run in triplicate. For 100% reference data three tablets were individually grinded with a mortar; the resulting powder was extracted with MeOH/DMSO (9 : 1) by ultrasonic and the solution was quantified by HPLC [7].

3.4. Partition coefficient

1 mg Hypericin alone or in combination with different amounts of flavonoids (hyperoside: 0.9, 4.6 or 9.2 mg; quercetin: 0.6, 3.0 or 6.0 mg; rutin: 1.2, 6.1 or 12.1 mg; procyanidin B2: 1.1, 5.7 or 11.5 mg) was dissolved in 2 ml n-octanol and partitioned with 2 ml water (aq. dem., pH 6.5). After shaking for 1 min the water phase was collected and centrifuged for 5 min at 4000 rpm. After addition of 5 µL of the internal standard solution to 1 ml of the received clear solution the sample was analysed by HPLC [7].

Acknowledgements: Thanks are due to Lichtwer Pharma AG, Berlin (Germany), for financial support; Dr. F. Petereit for a gift of procyanidin B2; Dr. M. Lechtenberg and Dr. J. Breitkreuz for helpful discussion; Prof. Dr. R. Gröning for the loan of the dissolution-apparatus; to Ms. B. Quandt and Mrs. U. Liefländer-Wulf for technical support.

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