Phytochemical composition and in vitro pharmacological activity of two rose hip (Rosa canina L.) preparations

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Abstract

The aim of the present study was to compare powdered rose hip with and without fruits (Rosae pseudofructus cum/sine fructibus, Rosa canina L., Rosaceae) with regard to their phytochemical profile and their in vitro anti-inflammatory and radical-scavenging properties. The two powders were subsequently extracted with solvents of increasing polarity and tested for inhibition of cyclooxygenase (COX-1, COX-2) and of 5-LOX-mediated leukotriene B4 (LTB4) formation as well as for DPPH-radical-scavenging capacity. While the water and methanol extracts were inactive in the COX-1, COX-2 and LTB4 inhibition assays, the n-hexane and the dichloromethane extracts inhibited all three enzymes. In the active extracts, the triterpenoic acids ursolic acid, oleanolic acid and betulinic acid were identified, although only in minute amounts. Furthermore, oleic, linoleic and α-linolenic acid were identified apart from several saturated fatty acids. Even though unsaturated fatty acids are known to be good inhibitors of COX-1, COX-2 and LT formation, no clear correlation between their concentration in the extracts and their activity was found. We suggest that other, yet unidentified, lipophilic constituents might play a more important role for the observed in vitro inhibitory activity on arachidonic acid metabolism. Some of the extracts also showed considerable DPPH radical scavenging activity, the methanolic extracts being most potent. The radical scavenging activity of the extracts correlated very well with their total phenolic content, while ascorbic acid contributes only little to the radical-scavenging activity due to its low concentration present in the extracts.

In summary, extracts derived from powdered rose hip without fruits were more effective in all assays carried out compared with extracts derived from powdered rose hip with fruits.

Keywords: Rosa canina L.; Rose hip; Rosae pseudofructus; Cyclooxygenase; Lipoxygenase; Anti-inflammatory; Radical-scavenging; DPPH; Triterpenoic acid

Introduction

Rose hip, the pseudo-fruit of Rosa canina L. (Rosaceae), consists of an urn-shaped receptacle with numerous achenes inside. Fresh rose hip is rich in vitamin C and is widely used for food production. Both fresh and dried rose
hips are frequently used as an ingredient of fruit and herbal teas. Concerning the use as a herbal drug, the plant part official in the European Pharmacopoeia is the rose hip without fruits (Ph. Eur., 2005).

Rose hip with or without fruits (Rosae pseudofructus cum/sine fructibus) is traditionally used for the prevention and therapy of common cold and other infections, as diuretic agent, for the treatment of various inflammatory diseases and as a vitamin C source. So far, for none of these indications clinical effectiveness has been demonstrated except for osteoarthritis (Blumenthal et al., 1998; Chrubasik et al., 2006). A rose hip and fruit powder, which is marketed as a food supplement in several European countries, has been shown to reduce osteoarthritis symptoms in clinical trials (Warholm et al., 2003; Rein et al., 2004). An anti-inflammatory mode of action has been suggested to explain the observed effects. This suggestion is corroborated by experiments in mice (Deliorman Orhan et al., 2007) and by in vitro data. A rose hip and fruit powder as well as a galactolipid isolated from this material have been shown in vitro to reduce chemotaxis of peripheral blood polymorphonuclear leucocytes and monocytes. These cells are involved in inflammatory processes and play a role for tissue damage in inflammatory diseases (Kharazmi and Winther, 1999; Larsen et al., 2003). A recent study also revealed an inhibitory effect of rose hip with fruit extracts on cyclooxygenase (COX)-1 and -2 in vitro (Jäger et al., 2007), and unsaturated fatty acids were suggested as active principle (Jäger et al., 2008).

Apart from the anti-inflammatory activity, an antioxidant mode of action might contribute to the observed clinical effects of rose hip preparations. Dæls-Rakotoarison et al. (2002) found that a rose hip acetone/water extract was able to scavenge reactive oxygen species. These radicals can lead to cell and tissue injury by reaction with biological materials, in cellular and acellular test systems.

The aim of this study was to compare two rose hip powders – one prepared from rose hip with fruits, and the other prepared from rose hip without fruits – with regard to their impact on arachidonic acid metabolism, their radical-scavenging potential and their phytochemical profiles.

Materials and methods

General experimental procedures

GC–MS measurements were performed on a HP 6890 GC–MS system (Agilent Technologies, Waldbronn, Germany) equipped with a J&W Scientific DB 225 column (30 m, i.d. 0.25 mm, film 0.25 μm; Agilent Technologies). Helium (0.8 ml/min) was used as a carrier gas, injector and detector temperatures were 220 °C, and the following temperature programme was used: 0–0.5 min 40 °C; 40–195 °C (25 °C/min); 195–202 °C (0.8 °C/min). Analytical HPLC measurements were performed on an Agilent 1100 series HPLC system (Agilent Technologies). LC–MS experiments were performed on a Thermo Finnigan Surveyor liquid chromatograph interfaced with a LCO™ Deca XP™PLUS mass detector. NMR spectra were recorded with a Varian® UnityInova (600 MHz) spectrometer using the parameters described by Seebacher et al. (2003). Pyridine-d5 was used as solvent and TMS as an internal standard; the experimental temperature was 40 °C.

Plant material and extraction

Two different starting materials were used for extract preparation: Rose hip fine powder, batch 119372 (Supplier Martin Bauer GmbH & Co Kg, Germany), containing rose hip without fruits (Rosae pseudofructus sine fructibus) for the RSF extracts and LitoZin®, batch 5141081 (Green medicine AB, Malmö, kindly provided by Nycomed, Switzerland), containing rose hip and fruits, for the RCF extracts, respectively. Litozin® is marketed as a food supplement in several European countries. Voucher specimens of the used materials are deposited at the herbarium of the Department of Pharmacognosy in Graz (RSF2007/1; RCF2007/1).

Both materials were subsequently soxhlet extracted with n-hexane, dichloromethane (DCM) and methanol. After methanol extraction the dried material was mixed with 10 fold amount of boiling water and stirred at room temperature for 1 h. After filtration, this step was repeated and the combined filtrates were concentrated and lyophilised. The organic extracts were concentrated under reduced pressure, and remaining solvent was removed under N2. The following extract yields were obtained: RSF n-hexane 0.9%, DCM 0.7%, methanol 21.0%, water 21.0%; RCF n-hexane 5.1%, DCM 0.5%, methanol 24.0%, water 11.2%.

In vitro assays for COX-1, COX-2 and LT formation inhibitory activity

COX-1 and COX-2 inhibition assays were performed in a 96-well-plate format with purified prostaglandin H synthase (PGHS)-1 from ram seminal vesicles for COX-1 and purified PGHS-2 from sheep placental cotyledons for COX-2 (both Cayman Chemical Company, Ann Arbor, USA) as previously described (Fiebich et al., 2005; Reininger and Bauer, 2006). The concentration of PGE2, the main arachidonic acid metabolite in this reaction, was determined by a competitive PGE2 EIA kit (Assay Designs Inc., Ann Arbor, MI, USA). Indomethacin (ICN, Aurora, USA; IC50 COX-1 0.9 μM) and NS-398 (Cayman
Chemical Company, IC\textsubscript{50} COX-2 2.6 μM) were used as positive controls.

The bioassay for inhibition of 5-LOX-mediated LTB\textsubscript{4} formation was carried out in a 96-well-plate format with stimulated human neutrophile granulocytes as described by Adams et al. (2004) with slight modifications (Knödler et al., 2008). Zileuton (Sequoia, Oxford, UK; IC\textsubscript{50} 5.0 μM) was used as positive control.

Test samples were dissolved in absolute ethanol. Extracts were tested at a final concentration of 50 μg/ml in the assay mixture, and pure compounds at 50 μM. Samples were tested in at least 3 independent experiments run in duplicate. Results are given as means±S.D. For comparison of inhibitory activity between RCF and RSF extracts, two-sided Mann–Whitney test was applied. Probability levels <0.05 were considered as statistically significant. Extracts and compounds showing inhibitory activity at the screening concentration were subjected to IC\textsubscript{50} determination by testing them in at least 3 concentrations in 3 independent experiments run in duplicate. Calculation of IC\textsubscript{50} values was performed by semilogarithmic presentation of dose vs. inhibitory activity and logarithmic regression analysis. The fatty acids tested in this study were purchased from Sigma (Steinheim, Germany), and the triterpene acids were the same as applied for HPLC studies.

DPPH radical scavenging assay (Schneider et al., 2004)

A volume of 50 μl of various methanolic dilutions of the rose hip extracts and of ascorbic acid were mixed with 150 μl of a 100 μM methanolic solution of DPPH. Each dilution was tested in triplicate. After 30 min the absorbances of the samples were read at 536 nm in a microplate reader. Quercetin (EC\textsubscript{50} 1.56 μg/ml) was used as a positive control.

Determination of total phenolic content

Determination was performed as described by Li et al. (2007). Samples were centrifuged (5’, 3500 rpm) prior to absorbance measurement. An external standard curve was prepared using gallic acid (10–250 μg/ml; Roth, Karlsruhe, Germany). Each determination was performed in triplicate.

Determination of ascorbic acid content

The ascorbic acid content of the extracts was determined by means of a Megazyme\textsuperscript{®} L-ascorbic acid assay (Megazyme International Ireland Ltd., Co. Wicklow, Ireland), according to the manufacturer’s instructions.

Analytical characterisation of the methanolic extracts

RCF and RSF methanolic extracts were subjected to solid phase extraction (SPE) to enrich the flavonoids. A total of 50 mg of extract was dissolved in 5 ml of MeOH/H\textsubscript{2}O 5:95 and the solutions were applied to preconditioned Isolute\textsuperscript{®} C18 500 mg/6 ml columns (International Sorbent Technology Ltd., Mid Glamorgan, UK). The columns were subsequently rinsed with MeOH/H\textsubscript{2}O 5/95, 30/70 and 70/30 (10 ml each). The 70/30 fraction was dried under reduced pressure and dissolved to 5 mg/ml in MeOH/H\textsubscript{2}O 70/30 for HPLC analysis. This was performed with a Zorbax SB C18 Narrowbore RR column (2.1 × 150 mm; 3.5 μm) and a Zorbax SB C8 guard column (2.1 × 12.5 mm; 5 μm) (Agilent Technologies). The mobile phase consisted of H\textsubscript{2}O (A) and MeCN/MeOH 4/3 (B), both containing 0.1% HCOOH. The following gradient was used: 0–20 min A:B 78:22–72:28; 20–35 min A:B 72:28–20:80; 35–45 min A:B 20:80; 45–60 min A:B 20:80–78:22; 46–55 min reequilibration. The flow rate was 250 μl/min, and the column temperature was 35°C. The same chromatographic method was used for LC-ESIMS\textsuperscript{™} analysis (capillary temp. 330°C; sheath gas flow 70 arbitrary units, auxiliary gas flow 10 arbitrary units; source voltage 4.5 kV; capillary voltage −38 V, tube lens offset 25 V).

Hyperoside, isoquercitrin and quercitrin and tiliroside were purchased from Roth (Karlsruhe, Germany). Methanolic solutions of these compounds were used for spiking experiments.

Determination of free fatty acids in the extracts showing in vitro anti-inflammatory activity

Free fatty acids were transformed to their respective methyl esters by derivatisation with BF\textsubscript{3}/MeOH as described previously (Liu et al., 1998) and quantified by GC–MS. Pentadecanoic acid (Sigma) was used as internal standard, and response factors for palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid were determined. The linearity range for each fatty acid was determined by injection of different dilutions ranging from 0.01 to 0.5 mg/ml and by subjecting the results to linear regression analysis (Bodensieck, 2008). All measurements were performed in triplicate and results are given as means±S.D.

Isolation of triterpenoic acids from the RSF DCM extract

A total of 3.4 g of RSF DCM extract was subjected to VLC on silica gel (0.04–0.063 mm, Merck, Darmstadt, Germany; 40 g) using a DCM/methanol gradient.
Fraction 6 (DCM/methanol 80/20, 400 mg) was further purified on a Sephadex® LH-20 column (3 x 50 cm), mobile phase DCM (ethyl acetate 1/1), and thereafter, 3 pure compounds were isolated by semipreparative HPLC on a LiChrosorb® RP 18 (7 μm) LiChroCART® 250-10 column (Merck, Darmstadt, Germany), using a gradient of water/acetonitrile 25/75-0/100 in 30 min (flow rate 2 ml/min). Compound 1 (RT 26.1 min, 1.9 mg) was identified as betulinic acid, compound 2 (RT 28.5 min, 3.0 mg) as oleanolic acid and compound 3 (RT 29.5 min, 1.9 mg) was found to be ursolic acid. Identification was accomplished by LC-APCI-MS and NMR spectroscopy (1H, HSQC, HMBC), and structures could be verified by comparison with literature data (Seebacher et al., 2003; Schüly, 2000).

**HPLC determination of the triterpenoic acid content**

Ursolic, oleanolic and betulinic acid content were determined in methanolic solutions of the RSF and RCF n-hexane and DCM extracts with a LiChrospher® 100 RP-18 (5 μm) LiChroCART® 250-4 column and a LiChrosorb® RP-18 (5 μm) LiChroCART® 4 guard column (Merck, Darmstadt, Germany). The mobile phase consisted of MeOH + 0.1% H₃PO₄ (77%), H₂O + 0.1% H₃PO₄ (18%) and MeCN (5%); after 45 min the column was rinsed for 5 min with MeOH + 0.1% H₃PO₄ (95%) and MeCN (5%) and reequilibrated for 10 min; the flow rate was 1 ml/min; detection wavelength was 205 nm and column temperature 20 °C. For calibration external standard curves were prepared with ursolic (Sigma), oleanolic and betulinic acid (Roth, Karlsruhe, Germany) solutions. Purity of the reference compounds was determined by HPLC using the system described above. Linearity of the method for each standard was established by injecting 10 μl of 7 different dilutions of the standard solution (50–1000 μg/ml) with 3 replicates. Coefficients of determination were found to be > 0.997. Intra-day precision and accuracy was determined by injecting 3 dilutions of a triterpene acid mixture on the same day (n = 5). These studies were repeated on 3 consecutive days to determine the inter-day precision and accuracy. Concerning intra-day precision, coefficients of variation were 1.92–4.32% for betulinic acid, 1.57–4.39% for oleanolic acid and 1.84–2.42% for ursolic acid; inter-day precision coefficients of variation were 1.14–4.06% for betulinic acid, 1.33–4.79% for oleanolic acid and 3.01–4.12% for ursolic acid. The accuracy (%) of the method, expressed as the mean deviation from the theoretical concentration, was 2.23–4.82% for betulinic acid, 0.21–4.93% for oleanolic acid and 1.18–4.78% for ursolic acid.

**Results and discussion**

**Impact of rose hip extracts on the arachidonic acid cascade**

At a screening concentration of 50 μg/ml the n-hexane and DCM extracts showed considerable inhibitory activity against COX-1, COX-2 and LT formation, while the water and methanolic extracts were inactive (Fig. 1). When comparing the n-hexane and DCM extracts of the two different rose hip preparations, the

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Fig. 1. COX-1, COX-2 and LT formation inhibitory activity of RCF and RSF extracts at a final concentration of 50 μg/ml assay mixture; mean (% inhibition)± S.D.; *p < 0.05; **p < 0.01 with two-sided Mann–Whitney Test when compared with the respective RCF extract.
RSF extracts showed significantly better activity in all three assays than the respective RCF extracts. For better comparability IC$_{50}$ values were determined (Table 1). The RSF hexane extract possessed the lowest IC$_{50}$ against COX-1, COX-2 and LT formation. IC$_{50}$ values of the RCF hexane extract were considerably higher concerning COX-2 and LT formation, and the IC$_{50}$ against COX-1 was found to be above the highest test concentration (125 $\mu$g/ml) and therefore not determined. The IC$_{50}$ values of the DCM extracts were higher in comparison to the $n$-hexane extracts, except for the IC$_{50}$ of the RCF extract in the LT assay. In a recent study, Jäger et al. (2007) showed that extracts from rose hip with fruit powder possessed pronounced in vitro COX-1 and COX-2 inhibitory activity. In contrast to our results, they found that the methanolic extract was more active than the $n$-hexane and DCM extract. We assume that this difference is caused by the fact that the authors did not extract the material subsequently with solvents of increasing polarity but prepared each extract individually. Therefore the results from that study cannot be directly compared with the present work.

Cyclooxygenases and 5-LOX are key enzymes of arachidonate metabolism which lead to the production of important mediators of inflammation. The observed inhibitory activity of lipophilic rose hip extracts against COX-1, COX-2 and 5-LOX-mediated LT formation indicates that rose hip preparations have an impact on arachidonate metabolism and that a powder containing rose hip without fruits might have a higher anti-inflammatory potential than a rose hip with fruit powder.

### Fatty acid determination in the active extracts and correlation of their fatty acid content to their in vitro anti-inflammatory activity

Rose hip is known to contain fatty acids such as palmitic, linoleic and $\alpha$-linolenic acid (Ercisli, 2007). It is also well known that unsaturated free fatty acids act as inhibitors of COX-1, COX-2 and LT formation in vitro (Liu et al., 1998; Reininger and Bauer, 2006). Jäger et al. (2008) report that unsaturated fatty acids are involved in the in vitro COX- inhibitory activity observed for rose hip extracts. In order to investigate the impact of the fatty acid content on the in vitro anti-inflammatory activity of rose hip extracts, the free fatty acid content of the active extracts was determined and set in correlation with the observed activity (Table 2, Fig. 2). In agreement with Ercisli (2007), palmitic acid, linoleic acid and $\alpha$-linolenic acid were identified as the main free fatty acids present in the lipophilic rose hip extracts, together with minor amounts of stearic acid and oleic acid. Depending on the starting material, the content of the fatty acids differed considerably: Palmitic acid and $\alpha$-linolenic acid were more abundant in the RSF extracts, while higher amounts of linoleic acid and oleic acid were found in RCF extracts. This finding might indicate that linoleic and oleic acid are more abundant in the fruits which are not contained in RSF.

Concerning the in vitro anti-inflammatory activity of the pure fatty acids, palmitic and stearic acid were found to be inactive in all three test systems, which is consistent with the literature (Liu et al., 1998; Reininger and

### Table 1. IC$_{50}$ values of the lipophilic RCF and RSF extracts in the COX-1, COX-2 and LT formation assay (water and methanol extracts showed no activity at the screening concentration)

<table>
<thead>
<tr>
<th>Extract</th>
<th>IC$_{50}$ COX-1 ($\mu$g/ml)</th>
<th>IC$_{50}$ COX-2 ($\mu$g/ml)</th>
<th>IC$_{50}$ LT assay ($\mu$g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSF</td>
<td>RCF</td>
<td>RSF</td>
</tr>
<tr>
<td>$n$-Hexane</td>
<td>22.9</td>
<td>&gt;125</td>
<td>10.4</td>
</tr>
<tr>
<td>DCM</td>
<td>35.4</td>
<td>&gt;125</td>
<td>29.2</td>
</tr>
</tbody>
</table>

### Table 2. Free fatty acid content (% of dry extract) of RCF and RSF $n$-hexane and DCM extracts, determined by GC–MS after derivatisation with BF$_3$/MeOH

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>% of $n$-hexane extract±S.D.</th>
<th>% of DCM extract±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSF</td>
<td>RCF</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>3.70±0.13</td>
<td>0.41±0.02</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>0.37±0.03</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.24±0.02</td>
<td>0.87±0.06</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1.16±0.06</td>
<td>3.50±0.25</td>
</tr>
<tr>
<td>$\alpha$-Linolenic acid</td>
<td>2.55±0.07</td>
<td>1.38±0.04</td>
</tr>
</tbody>
</table>
For linoleic acid the IC₅₀ values for COX-1, COX-2 and LT formation were found to be 14.0, 0.5 and 7.9 μM, respectively (Bodensieck, 2008) and for ω-linolenic acid 12.4, 5.4 and 15.3 μM, respectively, indicating considerable activity in all three assays. Oleic acid was only active in the LT formation assay (IC₅₀ 10.4 μM).

When we matched the fatty acid concentration present at the IC₅₀ of the extracts to the IC₅₀ values found for the pure compounds (Fig. 2), it turned out that fatty acids only play a minor role for the observed COX-1 inhibitory activity as their concentration in the assay mixture is far below the IC₅₀ of the respective pure fatty acids. For inhibition of LT formation, the presence of linoleic and ω-linolenic acid seems to play a more important role for the activity of RCF extracts than of RSF extracts. Concerning the influence of the fatty acids in the COX-2 assay, no consistent results were obtained: on one hand, linoleic and ω-linolenic acid seem to highly contribute to the COX-2 inhibitory activity of the active extracts. On the other hand, the RCF hexane extract was much less active than the RSF hexane extract, even though it contained much higher amounts of linoleic acid (about the 20 fold amount of the IC₅₀ found for the pure compound). The reason for this observation cannot be explained so far, but interaction with other constituents present in the extracts might play a role.

**Identification of triterpenoic acids in the active extracts, and correlation of the triterpene acid content to the in vitro anti-inflammatory activity**

LC-APCI-MS analysis of the active extracts indicated the presence of triterpene acids (data not shown). In order to achieve unambiguous identification, the RSF DCM extract, which seemed to be most concentrated in triterpene acids, was fractionated, and three pure compounds were isolated and identified as ursolic, oleanolic and betulinic acid. These compounds were isolated from rose hips for the first time.

These triterpenes are widespread in the plant kingdom and are known to possess various pharmacological activities. Ursolic and oleanolic acids were shown to have hepatoprotective, anti-inflammatory, antitumor and antihyperlipidemic effects in vitro and in vivo (Liu, 1995, 2005; Safayhi and Sailer, 1997). Also for betulinic acid a variety of biological activities are known, including inhibition of human immunodeficiency virus, antibacterial, antimalarial, anti-inflammatory, antihelminthic and antioxidant properties (Yogeesswari and Sriram, 2005). All three compounds have been shown to act as moderate inhibitors of COX-1, COX-2 and LT formation in different in vitro models (Najid et al., 1992; Ringbom et al., 1998; Santos Rosa et al., 2007). This was confirmed by our own experiments. IC₅₀ values determined for the three compounds are shown in Table 3.
In order to be able to estimate the contribution of these compounds to the activity observed for the rose hip extracts, their ursolic, oleanolic and betulinic acid content were determined by HPLC analysis. For both materials a higher triterpene acid content was found in the DCM compared with the n-hexane extracts. Betulinic and oleanolic acid were more abundant in the RSF than in the RCF extracts. Ursolic acid concentration was slightly higher in the RCF than in the RSF DCM extract (Table 4). From these data the concentration of ursolic, oleanolic and betulinic acid present in the assay mixture at the IC$_{50}$ of the extracts in the COX-1, COX-2 and LT formation assay were calculated. These concentrations were found to be 0.93–7.17 μM for betulinic acid, 0.17–1.32 μM for oleanolic acid and 0.07–0.93 μM for ursolic acid, which is far below the IC$_{50}$ values determined for the pure compounds (Table 3). Also mixtures of the three compounds tested at these concentrations did not show inhibitory activity in any of the assays. From these findings it can be concluded that the concentration of the triterpenoic acids identified in the active rose hip extracts is too low to significantly contribute to the observed COX-1, COX-2 and LT formation inhibitory activity.

**DPPH radical-scavenging activity of the extracts and its correlation to their total phenolic and ascorbic acid content**

For the RSF extracts the radical scavenging activity ranged from EC$_{50}$ values of 13.7 μg/ml for the methanolic extract to 364 μg/ml for the aqueous extract. The n-hexane and DCM extracts showed intermediate radical-scavenging activity. The activity of the RCF extracts was less pronounced, ranging from EC$_{50}$ values of 25.0 μg/ml for the methanolic extract to 988 μg/ml for the aqueous extract. As for both materials the methanolic extracts, for which no inhibitory activity had been observed in the COX-1, COX-2 and LT formation assays, were found to possess the highest DPPH radical scavenging activity, it can be concluded that this effect is caused by different active principles than the in vitro anti-inflammatory activity. In order to investigate the contribution of phenolic constituents to the observed activity, the total phenolic content of the extracts was determined and set in correlation with their DPPH radical-scavenging capacity (Table 5, Fig. 3). A very good correlation was found (coefficient of determination 0.9773). In addition to this, the contribution of ascorbic acid to the observed DPPH radical-scavenging capacity was investigated. The ascorbic acid concentration present at the EC$_{50}$ of the extracts was calculated and compared with the EC$_{50}$ of pure ascorbic acid (Table 6). No ascorbic acid was detected in the n-hexane and DCM extracts, and the ascorbic acid concentration determined for the aqueous and methanolic extracts was far below the EC$_{50}$ found for the pure compound. From these results it can be concluded that the phenolics present in the extracts play a prominent role for the observed DPPH radical-scavenging activity, while ascorbic acid does not significantly contribute to this effect. This is also corroborated by the findings of Daels-Rakotoarison et al. (2002), who showed that the in vitro ROS-scavenging activity of a rose hip water/acetone extract is not mediated by vitamin C but by phenolic constituents.

**Table 3.** IC$_{50}$ values (μM) determined for ursolic, oleanolic and betulinic acid in the COX-1, COX-2 and LT formation assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ COX-1 (μM)</th>
<th>IC$_{50}$ COX-2 (μM)</th>
<th>IC$_{50}$ LT assay (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ursolic acid</td>
<td>30.5</td>
<td>42.0</td>
<td>41.33</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>28.6</td>
<td>63.1</td>
<td>70.31</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>&gt; 125</td>
<td>&gt; 125</td>
<td>102.2</td>
</tr>
</tbody>
</table>

**Table 4.** Triterpene acid content (% of dry extract) of RCF and RSF n-hexane and DCM extracts, determined by HPLC

<table>
<thead>
<tr>
<th>Triterpene acid</th>
<th>% of n-hexane extract ± S.D.</th>
<th>% of DCM extract ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSF</td>
<td>RCF</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>4.09 ± 0.07</td>
<td>0.46 ± 0.00</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>0.76 ± 0.01</td>
<td>–</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>0.29 ± 0.01</td>
<td>–</td>
</tr>
</tbody>
</table>
Analytical characterisation of the methanolic extracts

The methanolic extracts which had shown the most prominent DPPH radical-scavenging activity were further characterised by HPLC analysis after SPE (Fig. 4). By analysing their MS fragmentation patterns and DAD spectra and by spiking the extract with authentic standards, hyperoside (peak 1), isoquercitrin (peak 2) and quercitrin (peak 4) could be unambiguously identified. Peak 3 was found to posses the molecular mass of a quercetin pentoside. The presence of hyperoside, quercitrin and various quercetin-pentosides in rose hip without fruits has also been reported by Hvattum (2002). The authors also report the presence of a quercetin-3-O-glucoside which they identified as isoquercitrin by LC–MS/MS.

Peaks 1–4 were detected in the flavonoid fractions of both the RCF and the RSF extract; however, they seem to be more abundant in the RSF extract. In the RCF extracts, however, two additional compounds (peaks 5 and 6) could be detected. Both compounds were found to possess molecular ions [M−H]− m/z 593 in the ESI negative mode and [M+H]+ m/z 595 in the ESI positive mode. MS2 in the negative mode yielded m/z 447 [M−H-coumaroyl]− and m/z 285 [M−H-coumaroyl-hexose]− as the main fragments. From these data, the two peaks could be assigned to kaempferol 3-O-(6′-O-E/Z-p-coumaryl)-glucosides (cis- and trans-tiliroside). The two compounds have previously been found to be present in the fruits of *R. canina* as a *trans:cis* mixture in a ratio of about 2:1 (Kumarasamy et al., 2003). Therefore, the more abundant peak was suggested to be *trans*-tiliroside. This could be confirmed by spiking with the authentic reference compound. The two isomers were predominant in the HPL chromatogram of the RCF extract, while they were only present in traces in the RSF extract. This is due to the fact that they are only contained in the fruits but not in the fleshy peels of *R. canina*. They might therefore be a good marker for the discrimination between rose hip preparations with and without seeds.

In summary, the phytochemical and pharmacological investigations of two rose hip preparations revealed pronounced *in vitro* anti-inflammatory and radical-scavenging activity of different rose hip extracts. Extracts derived from the powder containing only rose hip without fruits (RSF) were more effective in all assays than extracts derived from rose hip with fruits (RCF) powder. This leads to the conclusion that the active principles responsible for these effects are more abundant in rose hip fleshy peels than in the fruits. Constituents with inhibitory activity on COX-1, COX-2 and LT formation have been shown to be enriched in the lipophilic extracts. As the observed activity could not clearly be correlated to the presence of unsaturated fatty acids and triterpene acids in these extracts we suggest the contribution of other, yet unidentified constituents or a synergistic effect causing the observed activity. The high correlation of DPPH radical-scavenging activity and total phenolic content of the extracts as well as the low vitamin C content detected in the extracts leads to the conclusion that this effect is mainly caused by phenolic constituents and not by vitamin C.

These findings, together with other data from literature, raise the question of appropriate standardisation of...
rose hip preparations. According to the European pharmacopoeia (Ph. Eur. 2005), rose hip is standardised on vitamin C. Also a commercially available rose hip and fruit powder which has shown to possess anti-inflammatory properties in vitro and in vivo (Kharazmi and Winther, 1999; Larsen et al., 2003) and to clinically reduce osteoarthritis symptoms (Warholm et al., 2003; Rein et al., 2004) is standardised to a vitamin C content of at least 500 mg/100 g powder. Furthermore, this product is stated to contain 57.9 mg/kg β-carotene and 4.6 mg/100 g vitamin E, apart from other constituents such as β-sitosterol, folic acid, magnesium, zinc and copper (Rein et al., 2004). Even though no contribution to the radical and ROS-scavenging activity was found for vitamin C in the present study and in the study of Daels-Rakotoarison et al. (2002), vitamins C, E and carotenes are known to possess antioxidant effects. However, vitamin C was shown not to be involved in the inhibition of chemotaxis in neutrophils (Kharazmi and Winther, 1999), and at present there is no clear evidence that vitamins C or E are clinically effective in the treatment of any type of arthritis (Canter et al., 2007). Therefore, standardisation of rose hip products on their vitamin C and E content alone might not be sufficient. Apart from vitamins C and E, a galactolipid isolated from a certain rose hip cultivar (Larsen et al., 2003) is also regarded as a possible active principle. However, so far, a chemotaxis inhibitory activity for this compound has only been shown in vitro, while in vivo or clinical effectiveness is not yet proven.

Therefore, further research concerning the active principles and the clinical effectiveness of rose hip will be necessary in order to allow appropriate standardisation and a rational use of rose hip preparations.

References


