Institute of Pharmaceutics and Biopharmaceutics[1], Institute of Pharmaceutical Chemistry[2], Department of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle, Germany

Antioxidant and photoprotective properties of an extract from buckwheat herb (*Fagopyrum esculentum* MOENCH)

I. Hinneburg[1], S. Kempe[1], H-H. Rüttinger[2], R. H. H. Neubert[1]

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Sabine Kempe, Institute of Pharmaceutics and Biopharmaceutics, Wolfgang-Langenbeck-Str. 4, D-06120 Halle
sabine.kempe@pharmazie.uni-halle.de

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In recent years, the incidence of skin cancer has risen remarkably. Sun light, especially the included ultraviolet (UV)-radiation, is seen as important trigger for the development of skin cancer. Thus, there is an increasing interest in the development of UV-protective substances to use them as sun care products. One approach is the topical application of herbal antioxidants. Plant-derived antioxidants are often extracts and therefore contain a complex mixture of constituents, like flavonoids and polyphenols, which contribute to the overall activity of the extract. In the present study an extract from buckwheat herb was compared to rutin, which is the main constituent of the extract, regarding their antioxidant and radical scavenging activity. Additionally, the photoprotective properties of the extract were compared to those of a commercial UV absorber. The antioxidant activity was quantified regarding the reactivity versus the 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH). The photoprotective properties of the extract were examined by the inhibition of the photosensitized lipid peroxidation of linolic acid. In the DPPH assay, the extract had significantly better antioxidant activity than pure rutin. The extract prevented more effectively the UV-induced peroxidation of linolic acid than rutin itself or the commercial UV absorber. The use of the extract from buckwheat herb seems to be more beneficial than the use of pure rutin. This can be referred to the presence of minor phenolic compounds in the extract. The results indicate that it is advisable to use antioxidants rather than only UV absorber to obtain a maximum of photo protection.

1. Introduction

During recent years, increasing knowledge was gained about the effects of ultraviolet radiation on human skin. UV radiation was claimed responsible for a variety of photobiological disorders, including photoaging and photocarcinogenesis (Pinnell 2003). The photodamage caused by UV radiation has been attributed to the formation of reactive oxygen species and subsequent oxidation processes to different targets in human skin as lipids, proteins and DNA (Yamamoto 2001).

Numerous approaches were proposed for the prevention of photodamage to human skin. Besides avoiding sunlight and the use of protecting clothes, the application of sun care products with high UV absorbing properties has been advised (Rosen 2003). Furthermore antioxidants as ascorbic acid and tocopherol were administered topically (Fuchs 1998). Especially antioxidants derived from plants have been screened for their usefulness in photochemoprevention. Among other substances flavonoids and related phenolic substances showed good results as inhibitors of lipid peroxidation and of DNA damage (F'guyer et al. 2003).

Buckwheat (*Fagopyrum esculentum* Moench) is traditionally used for venous diseases (Ihme et al. 1996). It has a high content of phenolic substances such as flavonoids and phenolic acids (Hagels et al. 1998). The main phenolics are rutin, chlorogenic acid and hyperoside. Rutin, whose content in buckwheat herb is about 5–8%, has the ability to absorb UV radiation and scavenge free radicals as the superoxide anion, hydroxyl radical and peroxyl radical (Rice-Evans et al. 1996). So its use in sun care products seems to be interesting. Hyperoside and chlorogenic acid have antioxidant activity as well (Azuma et al. 1999). Because flavonoids are considered to be effective absorbers of UV radiation in plants (Kreft et al. 2002), this property can additionally contribute to the usefulness of plant extracts in sun care products.

Plant extracts are complex mixtures of several compounds. Extracts examined from Eder and Mehnert (1998) had additional benefits compared to single compounds regarding the solubility or the absorption. Niesel (1992) found a five times better solubility of rutin in buckwheat herb than for the isolated substance.

The aim of this work was to investigate antioxidant and radical scavenging properties of an extract from buckwheat herb in comparison to pure rutin. Additionally, the photoprotective activity of the extract was compared to these of a commercial UV absorber.

Table: Phytochemical characteristics of the extract from buckwheat herb

Drug extract ratio	3.57 – 4.55 : 1
Rutin (%)	19.54 – 22.31
Hyperoside (%)	0.18 – 0.37
Chlorogenic acid (%)	4.09 – 5.57

Data are given as the range of seven batches. The content of phenolics was determined by capillary electrophoresis as described in Hinneburg et al. (2004)

2. Investigations, results and discussion

2.1. Characterization of the extract

The phytochemical characteristics of the extract yielded from buckwheat herb by a maceration process are given in the Table.

Theurer et al. (1997) found phototoxicity for extracts from buckwheat herb which has been referred to the naphthodianthrone fagopyrin (Theurer et al. 1997). The extraction method has been optimized to reduce the fagopyrin content in the extract, in order to avoid this problem (Hinneburg and Neubert 2005). Additionally, the absence of fagopyrin in the extract has been confirmed by TLC (data not shown).

2.2. Radical scavenging activity on DPPH

In the DPPH assay, the extract and equivalent amounts of pure rutin were compared regarding their radical scavenging activity. DPPH, a stable radical, changed its colour from violet to yellow upon reduction (Brand-Williams et al. 1995) and therefore indicated the antioxidant activity of the tested compound.

The antioxidant activity of both substances, extract and rutin, increased in a dose dependent manner (Fig. 1). The difference between the antioxidant activity of the extract and rutin was significant up to a concentration of 20 μg/ml ($p < 0.01$). The calculated SC_{50} values were 16.67 ± 1.11 μg/ml for rutin and 59.01 ± 5.93 μg/ml for the extract. Refering to the rutin content of the extract (20%), the SC_{50} value was only 11.87 ± 1.24 μg/ml and therefore significantly lower than the corresponding value for rutin ($p < 0.01$).

Our results indicated differences between the extract and pure rutin although they were assayed in the same concentrations (calculated for rutin). This could be referred to other phenolic substances in the extract which contributed to the overall antioxidant activity of the extract. In comparison to other plant extracts the determined SC_{50} value for the extract is rather low (Mensor et al. 2001; Dorman et al. 2004). Therefore the extract from buckwheat herb can be considered as a very good antioxidant.

2.3. Photoprotective activity in the test system of ketoprofen-sensitized peroxidation of linoleic acid

A second system was chosen to study the antioxidant activity of buckwheat extract, because DPPH is only a model radical and has no physiological relevance. Therefore ketoprofen (KP) was used as a photosensitizer to generate free radicals by UVA irradiation. These radicals peroxidize linoleic acid (LA) which is a part of the skin lipids. We compared the activity of the extract, pure rutin and the commercial UV filter substance Uvinul MS 40. Buckwheat extract had two benefits in our model system: it decreased the photodegradation of KP by absorbing UVA radiation and it decreased the formation of linoleic acid peroxides (HPODE) by interfering with the initiation and propagation of the lipid peroxidation.

Fig. 2 shows that buckwheat extract decreased the photodegradation of KP in a dose dependent manner. The highest inhibition was obtained with a concentration of 1.25 mg/ml. At a dose level of 5 J/cm^2 UVA the KP degradation rate was significantly different for each concentration ($p < 0.01$). Furthermore buckwheat extract inhibited the peroxidation of linoleic acid. (Fig. 3) The levels of HPODE were significantly lower ($p < 0.001$) with each investigated concentration of buckwheat extract compared to irradiated control. Buckwheat extract also decreased more significantly the level of HPODE than equivalent amounts of rutin ($p < 0.01$). No significant difference between the different concentrations of the extract could be observed in the formation of HPODE, whereas rutin decreased the amount of HPODE in a dose-dependent manner. Even at the lowest concentration of 0.3 mg/ml of buckwheat extract, there was only a minimal formation of HPODE in this assay.

Within the model system it was possible to distinguish to which extent the reduction of HPODE formation could be attributed to the UV absorbing properties as well as to the antioxidant activity of the extract. Therefore, the experiments were repeated using a commercial UV filter substance, Uvinul MS 40, in amounts corresponding to the absorption properties of the used concentrations of buck-

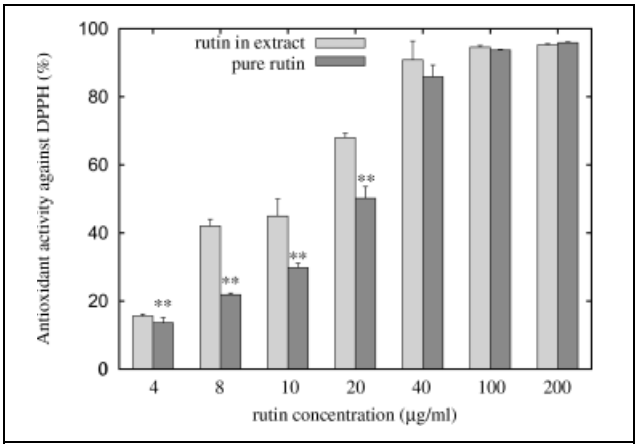

Fig. 1: Antioxidant activity of rutin and extract versus the DPPH radical. Data are given as means ± standard deviations (n = 3). **: significant difference from extract values ($p < 0.01$)

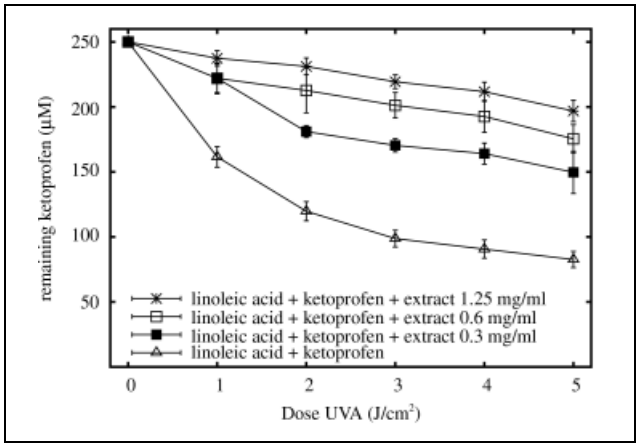

Fig. 2: Photodegradation of ketoprofen induced by UVA irradiation under the influence of buckwheat extract. Data are given as means ± standard deviations (n = 3)

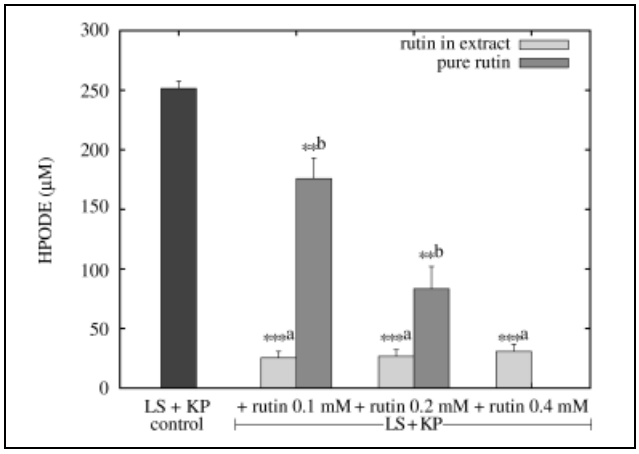

Fig. 3: Increase of HPODE under the influence of buckwheat extract and pure rutin at an irradiation level of 5 J/cm^2 UVA. Data are given as means ± standard deviations (n = 3). LA = linoleic acid, KP = ketoprofen. ***a: significant difference from irradiated control ($p < 0.001$), **b: significant difference from extract values ($p < 0.01$)

wheat extract. The amounts were calculated from the integrated absorptivity in the UVA region, so that the absorption of Uvinul MS 40 corresponded to the absorption of the extract in respective concentrations.

Uvinul MS 40 decreased ketoprofen photodegradation in a dose-dependent manner. In the case of equal absorbance, the degradation of ketoprofen should be similar in the presence of corresponding concentrations of Uvinul MS 40 and the extract. In fact, in experiments no significant difference in the degradation of ketoprofen could be observed for either concentration (data not shown).

Uvinul MS 40 inhibited the formation of HPODE depending on the concentration used. (Fig. 4) If the extract had only UV absorbing quality and no antioxidant activity, the increase of HPODE of the extract and Uvinul MS 40 should both yield the same. But a significantly higher inhibition in the formation of HPODE for the extract than for Uvinul MS 40 was observed. (Fig. 4) This led to the conclusion that the antioxidant activity of the extract contributed significantly to the retention of linoleic acid peroxidation, besides UV absorption.

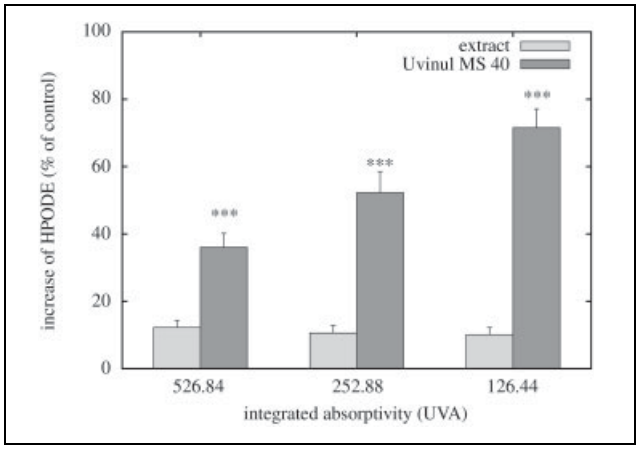

Fig. 4: Comparison of the influence of buckwheat extract and an UV absorber on the formation of HPODE at an irradiation level of 5 J/cm^2 UVA. The integrated absorptivity (IA) corresponds to the following concentrations of buckwheat extract (BE) and Uvinul MS 40 (UMS): 126.44 IA b = 0.3 mg/ml BE b = 0.21 mg/ml UMS; 252.88 IA b = 0.6 mg/ml BE b = 0.42 mg/ml UMS; 526.84 IA b = 1.25 mg/ml BE b = 0.89 mg/ml UMS. Data are given as means ± standard deviations (n = 3). ***: significant difference from extract values ($p < 0.001$)

2.4. Conclusions

We demonstrated that the extract from buckwheat herb is a potent antioxidant. Besides rutin, the main phenolic compound, the extract contains other flavonoids and phenolic acids which contribute to the overall antioxidant activity of the extract. Thus, the extract reacted better in a radical scavenger system than corresponding amounts of pure rutin. Lipid peroxidation was inhibited more effectively by the extract than by a commercial UV filter. In conclusion it is more beneficial to use an extract from buckwheat herb than pure rutin. The results indicate as well, that the combination with antioxidants provides a better photoprotection in terms of lipid peroxidation than mere UV filters alone. However, the absence of phototoxicity still has to be demonstrated *in vivo*. The development of semi-solid formulations to apply the extract as a topical antioxidant as well as the stability of the extract are currently under investigation.

3. Experimental

3.1. Materials

Chemicals and reagents were obtained from the following commercial sources: Ketoprofen (Bayer, Leverkusen, Germany), sodium borate (Merck, Darmstadt, Germany), Uvinul MS 40 (BASF, Ludwigshafen, Germany), rutin (Acros Organics, Geel, Belgium), linoleic acid, 13(*S*)-hydroperoxy-octadeca-9*Z*,11*E*-dienoic acid (13-HPODE) and DPPH (Sigma, Deisenhofen, Germany). All samples, solutions and buffers were prepared from double distilled water. The buckwheat extract was prepared from dried buckwheat herb (Caelo, Hilden, Germany) by macerating in ethanol 30% (v/v) at 60 °C for 2 h and subsequent freeze-drying, as described by Hinneburg and Neubert (2005).

3.2. DPPH assay

The freeze-dried extract and rutin were dissolved in methanol at a concentration of 1 mg/ml. DPPH concentration in methanol was 100 µg/ml. Measurement were performed in microtiter plates according to Fukumoto and Mazza (2000).

100 µl extract solution were mixed with 100 µl DPPH and the absorption at 540 nm was recorded after 10 min (microtiter plate reader Polar Star Galaxy, BMG Labtechnologies, Offenburg, Deutschland). The control was a mixture of 100 µl DPPH and 100 µl methanol. Antioxidant activity (AA) was calculated according to the following equation:

$$AA(\%) = 100 \cdot (A_c - A_s)/A_c$$

where A_s is the absorbance of the sample and A_c is the absorbance of the control. The measurements were performed in triplicate.

3.3. Ketoprofen-induced photoperoxidation of linoleic acid

3.3.1. Preparation of solutions

5 ml of a 2 mM linoleic acid solution (in 10 mM sodium borate solution) were mixed with 2.5 ml of a 1 mM ketoprofen solution (in phosphate buffer pH 7.0) and either 2.5 ml of phosphate buffer pH 7.0 or a solution of buckwheat extract (in 10 mM sodium borate solution) in appropriate concentrations were added, resulting in the following concentrations: linoleic acid 1 mM, ketoprofen 0.25 mM and buckwheat extract 0.3, 0.6 and 1.25 mg/ml respectively. The chosen amounts of buckwheat extract correspond to 0.1 mM, 0.2 mM and 0.4 mM rutin.

3.3.2. Calculation of integrated absorbance area

UV spectra of solutions of the extract and Uvinul MS 40 were recorded with a photospectrometer M 500 (Analytik Jena, Jena, Germany) in the range of 200 to 400 nm.

The area under the curve in the range between 320 and 400 nm (corresponding to the UVA region) was calculated by integration (Origin 6.1, OriginLab, Northampton, USA). To obtain the specific absorption area, the value for the area was divided by the concentration of the solution. From the specific absorption area, the amount of Uvinul MS 40 needed was calculated to obtain the same absorbance area for the used concentrations of buckwheat extract solution in the irradiation experiments. The concentrations of Uvinul MS 40 corresponding to the UVA absorption of buckwheat extract were calculated as 0.21 mg/ml, 0.42 mg/ml and 0.89 mg/ml.

3.3.3. Irradiation

The solutions prepared as described above were irradiated in glass beakers (diameter 5.1 cm) covered with fused silica plates under permanent stirring. The irradiation doses were 0–5 J/cm^2 UVA. The irradiation sources were Philips CLEO Performance R UVA lamps (305–420 nm) from Veith Import-Export (Westerau, Germany). The UV doses were measured with UV sensors from Kühnast Strahlungstechnik (Wächtersbach, Germany). After irradiation of a sample 50 µl were taken and analyzed by capillary electrophoresis (CE). All experiments were performed in triplicate.

3.3.4. CE analysis conditions

A CE system with UV detection from Dionex (Dionex GmbH, Idstein, Germany) was used (Radschuweit et al. 2001). Fused silica capillaries (45 cm × 75 µm, 40 cm to detector window) were purchased from Supelco (Deisenhofen, Germany). Samples were introduced by gravity injection from 20 cm height for 20 s. The simultaneous detection of ketoprofen, ketoprofen degradation products, linoleic acid peroxides and rutin was performed at 234 nm and a voltage of 25 kV. The ground electrolyte was 0.01 M sodium borate. Ketoprofen and 13-HPODE were used as standards for the recording of calibration curves.

3.4. Statistics

All data are given as means ± standard deviations. Statistical significance was determined by analysis of variance after logarithmic transformation of the data and Newman-Keuls post test. P values equal or less than 0.05 were considered significant (Graph Pad Prism 2.0, GraphPad Software Inc., San Diego, USA). SC_{50} values were calculated from non-linear regression using Origin 6.1 (OriginLab, Northampton, USA).

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