Watercress – cultivation methods and health effects

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Summary
Watercress, Nasturtium officinale R. Br., is a native water or semi-aquatic plant that has a high nutrient density. Physiologically relevant are the various glucosinolates, which possess positive health effects in form of their thio- and isothiocyanates. In an interdisciplinary project, we aim to develop a hydroponic, and finally an aquaponic, circulatory cultivation system and to study the health effects of watercress. In humans, there is a lack of data-based knowledge on potential beneficial health effects of watercress. Growth of watercress was followed during one season in an open-door hydroponic system. Watercress was also cultivated in the greenhouse in different substrates with different concentrations of nutrients and salt. The biomass production is strongly dependent on the temperature. The glucosinolate contents differ significantly during the growing season, especially during flowering. Watercress naturally grows in nutrient-rich fresh waters, however, when cultivated at NaCl concentrations of up to 120 mM the gain in biomass is still high. In a human proof-of-concept study, indications for antioxidant and anti-inflammatory effects of fresh watercress were observed already after a single dose intake of fresh watercress (85 g). Further in vivo and in vitro studies are planned to study health beneficial effects of watercress and its metabolic activity.

Keywords: Anti-inflammatory, antioxidative, gluconasturtiin, glucosinolates, hydroponic cultivation, PEITC.

Introduction
Watercress (Nasturtium officinale R. Br.), a member of the Brassicaceae, is a perennial aquatic or semi-aquatic plant species native to Europe and Asia. Watercress grows in nutrient-rich, streaming freshwater (Kopsell et al., 2007). Watercress is traditionally used as winter salad as it grows in flowing water even at cool temperatures as long as the water is not frozen. Due to its special demands, the cultivation of watercress declined although nutritionally valuable metabolites have been identified. Usually, watercress is cultivated in sophisticated held back streaming waters, but also grows well in moist soil or hydroponic cultures. When commercially grown, watercress cuttings or seedlings are planted into beds with a mixture of soil and gravel, leveled out to ensure even water flow through the beds. Upper parts of the watercress are harvested several times per growing season, leaving enough stem to ensure new growth (Tab. 1). The species needs low amounts of nitrogen and phosphate in comparison to other plant species while producing large amounts of biomass (Kopsell et al., 2007). As it is quite low in energy, watercress has a high nutrient density for vitamins B1, B2, B3, B6, E, C, polyphenols (flavonoids, phenolic acids, proanthocyanidins) as well as terpenes (including carotenoids) (Klimek-Szczytkowicz et al., 2018). Like all members of the Brassicaceae plant species watercress contains mustard oil glycosides or glucosinolates (GLS). These nitrogen and sulfur containing secondary metabolites are derived from amino acids and are synthesized by the plant to cope with biotic stressors. Glucosinolates and thioglucosidases (EC 3.2.1.147) are usually stored in different cells or cell compartments, but get together once the plant tissues are disrupted (Ahuja et al., 2016). Thioglucosidases then hydrolyze the GLs leaving an unstable aglucone behind, which further reacts to thiocyanates, isothiocyanates and nitriles depending on pH, metal ions and present specifier proteins (Chen et al., 2019). In the case of watercress, the eponymous GL gluconasturtiin predominates, a precursor of the breakdown product phenethyl isothiocyanate (PEITC). Isothiocyanates and thiocyanates are very reactive substances leading to numerous conjugates with thiol containing compounds like N-acetylcysteine, glutathione, cysteine and many more, forming stable thiocarbamates (Müller et al., 2018). Several health beneficial effects have been postulated for watercress. These include antioxidant, anti-inflammatory, immunomodulating, anti-diabetic, anti-allergic, antibacterial, hypolipemic, cardioprotective and anticancer effects as well as beneficial effects on the reproductive system (summarized in Tab. 2-4). Most of these effects have been observed in vitro (Tab. 2) and in animal studies (Tab. 3), while only a few human intervention studies have been carried out (Tab. 4). However, findings from in vitro studies do not necessarily apply in vivo, especially when looking at antioxidant effects of compounds (Berger et al., 2012). Although some studies analyzed the administration of isolated PEITC (Yuan et al., 2016), which is the main isothiocyanate of watercress, the investigation of single compounds does not necessarily allow drawing conclusions from the effects of whole watercress – an edible green with other known health-promoting ingredients.

Overall, the data on the nutritional effects of watercress is very limited. The few human studies that administered watercress focused on

Tab. 1: Cultivation methods of watercress.

<table>
<thead>
<tr>
<th>Cultivation method</th>
<th>Place</th>
<th>Substrate used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beds with flowing water</td>
<td>Germany (Erfurt)</td>
<td>Soil mixed with gravel</td>
<td>Pink, 1993</td>
</tr>
<tr>
<td>Beds with flowing water</td>
<td>Great Britain (Dorset, Hampshire)</td>
<td>Soil mixed with gravel</td>
<td>Casey &amp; Smith, 1994; Crisp, 1970</td>
</tr>
<tr>
<td>Beds with flowing water</td>
<td>USA (California, Hawaii, Florida)</td>
<td>Soil or sand</td>
<td>Fennell, 2006</td>
</tr>
<tr>
<td>Hydroponics or overhead spray lines</td>
<td>Australia (Brisbane, Sydney, Melbourne)</td>
<td>Nutrient solution</td>
<td>Fennell, 2006</td>
</tr>
</tbody>
</table>

¹ Corresponding author
antioxidant (Fogarty et al., 2013; Gill et al., 2007) and anticancer effects (Hoffmann et al., 2009), while anti-inflammatory effects have not been investigated in humans so far. In an interdisciplinary research project, we aim to optimize the cultivation of watercress in aquaponic circulatory systems. Another aim of the project is to study the health effects of freshly harvested watercress in vivo. A proof-of-concept study was conducted to prove the applicability of a specific study design to investigate antioxidative and anti-inflammatory effects of watercress in humans. The effect of a single watercress dose on markers of oxidative stress/lipid peroxidation (malondialdehyde, MDA) and inflammation (IL-6, TNFα and IL-10) was investigated in subjects who had to complete a high-intensity training to induce oxidative stress and a pro-inflammatory condition.

Tab. 2: Health effects of watercress – in vitro studies.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Dosage form</th>
<th>Cell line/ experimental model</th>
<th>Results/Mechanism(s)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Anticancer                            | Extract of watercress | Human MDA-MB-231 breast cancer cells | • Suppression of invasive potential  
• Inhibition of metallo-proteinase 9                                                                                   | Rose et al., 2005           |
| Anticancer                            | Extract of watercress | Human HT115 colon cancer cells | • Suppression of invasive potential  
• Reduction in cisplatin resistance  
• Increased rate of apoptosis of cancer cells  
• Inhibited xenograft tumor growth                                                                                   | Boyd et al., 2006          |
| Anticancer                            | PEITC       | Biliary tract cancer cells    | • Watercress extract proved to be effective against tumor initiation, proliferation and metastasis:  
• Inhibition of DNA damage (initiation)  
• Accumulation of cells in the S phase of the cell cycle (proliferation)  
• Inhibition of invasion through matrigel (metastasis)                                                               | Boyd et al., 2006          |
| Antioxidant, hypolipidemic and cardipo-\protective | Extracts of watercress | Rat liver homogenate          | • Reduced serum alanine aminotransferase and aspartate aminotransferase levels compared to high-fat diet groups  
• Reducing power in a ferric reducing antioxidant power assay  
• Concentration-dependent scavenging ability on 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonate, 1,1-diphenyl-2-picyrylhydrazyl, nitric oxide radicals, and hydrogen peroxide  
• Chelating ability on ferrous ions  
• Prevention of thiobarbituric acid reactive substances formation in ferrous ion/ascorbate induced lipid peroxidation in a dose dependent manner | Bahramikia and Yazdanparast, 2010 |
| Antioxidant and antidiabetic           | Watercress juice | Digestive enzymes: α-glucosidase, α-amylase and lipase | • Inhibition of α-glucosidase, α-amylase and lipase                                                           | Spinola et al., 2017       |
| Antioxidant                           | Extract of watercress (aqueous and ethanol) | Direct measurement of antioxidant capacity of watercress extract | • Improved total antioxidant activity, reducing power, DPPH* radicals and superoxide anion radicals scavenging activities | Ozan, 2009                 |
| Antioxidant and anticancer             | Extract of watercress & PEITC | Human PBMC                    | • Increased gene expression of detoxification enzymes (GPx1 and SOD2)  
• Increased SOD2 activity                                                                                           | Hoffmann et al., 2009      |
| Anti-inflammatory                     | PEITC       | Murine raw 264.7 macrophages  | • Inhibition of NO production → decreased production of TNFα and IL-10 by activated macrophages  
• Increased NO clearance                                                                                              | Tsai et al., 2010          |
| Antiallergic                          | Extract of watercress (ethanol) | Rat peritoneal mast cells and rat basophilic leukemia cells (RBL-2H3) | • Inhibition of histamine release                                                                               | Hoshino et al., 1998      |
| Antibacterial                         | Extract of watercress (methanol) | Gramnegative bacteria (e.g. Escherichia coli, Klebsiella pneumoniae) and Grampositive bacteria (e.g. Enterococcus faecalis and Bacillus cereus) | • Antibacterial activity for all bacterial strains  
• Highest inhibitory activity against Bacillus cereus and Escherichia coli                                             | Zafar et al., 2017         |
| Antibacterial                         | Extract of watercress (chloroform) | Mycobacterium tuberculosis H37Rv bacteria | • Inhibitory activity against Mycobacterium tuberculosis H37Rv bacteria                                                                 | Quezada-Lázaro et al., 2016 |

DPPH, 2,2-diphenyl-1-picrylhydrazyl; GPx1, glutathione peroxidase 1; IL-10, Interleukin 10; NO, nitric oxide; PEITC, phenethyl isothiocyanate; SOD, superoxide dismutase; TNFα, tumor necrosis factor α.
Materials and methods

Plant material

The watercress cultivar was originally obtained from the nursery Fischer, Erfurt (http://erfurt-hochheim.de/gewerbe_und_handel/handel/?id=75). It was further propagated on the trout farm of the family Göckemeyer in Poggenhagen (http://www.edelkrebs-niedersachsen.de/forellen/). There, cuttings have been taken for propagation in the greenhouse at the Institute of Botany, Leibniz University Hannover.

Sampling and cultivation experiments

Growth of watercress was followed during one growth season in an outdoor hydroponic system and samples were collected every two

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Tab. 3: Health effects of watercress – animal studies.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Dosage form</th>
<th>Species</th>
<th>Results/Mechanism(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer</td>
<td>Aqueous solution of watercress</td>
<td>Swiss mice</td>
<td>• Suppression of Ehrlich tumor growth</td>
<td>DE SOUZA et al., 2016</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Extract of watercress</td>
<td>Rats</td>
<td>• Inhibition of carrageenan-induced paw edema</td>
<td>SADEGH et al., 2014</td>
</tr>
<tr>
<td>Antioxidative, hypolipemic and cardioprotective</td>
<td>Extract of watercress (Hypercholesterolaemic) rats</td>
<td></td>
<td>• Decrease of hepatic MDA, GR and GPx activities</td>
<td>YAZDANPARAST et al., 2008</td>
</tr>
<tr>
<td>Antioxidant and anti-inflammatory</td>
<td>Extract of watercress</td>
<td>Rats</td>
<td>• Protection against increase in ROS, GSH, LPO and PCO in gentamicin-induced nephrotoxicity</td>
<td>SHAHANI et al., 2017</td>
</tr>
<tr>
<td>Antioxidant and antidiabetic</td>
<td>Extract of watercress (Diabetic) rats</td>
<td></td>
<td>• Improvement of antioxidant status: SOD, GR, GPx, MDA in plasma and different tissues, total antioxidant status</td>
<td>FENTON-NAVARRO et al., 2018</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Different extracts of watercress (ethyl acetate, methanol and aqueous)</td>
<td>(Diabetic) rats</td>
<td>• Decrease of blood glucose after 1 week and 2 months</td>
<td>HOSEINI et al., 2009</td>
</tr>
<tr>
<td>Antidiabetic and hypolipidemic</td>
<td>Extract of watercress (hydro-alcoholic)</td>
<td>(Diabetic) rats</td>
<td>• Decrease of serum glucose, total cholesterol and LDL-cholesterol</td>
<td>HADIZADEH et al., 2015</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Extract of watercress (hydro-alcoholic)</td>
<td>Rats</td>
<td>• Attenuation of Vancomycin-induced nephrotoxicity</td>
<td>KARAMI et al., 2018</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Watercress juice</td>
<td>Mice</td>
<td>• Improvement of superoxide dismutase activity in erythrocytes</td>
<td>CASANOVA et al., 2017</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Extract of watercress (ethanolic)</td>
<td>Rats</td>
<td>• Decreased lipid peroxidation in liver, brain and kidney</td>
<td>OZEN, 2009</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Watercress oil</td>
<td>Rabbits</td>
<td>• Improved SOD activity and GSH concentrations</td>
<td>ALAGAWANY et al., 2018</td>
</tr>
<tr>
<td>Immuno-modulating</td>
<td>Extract of watercress</td>
<td>Rainbow trout</td>
<td>• Enhancement of hematological and immunological parameters (including Hb and MCHC, lysozyme and complement activities, total protein and globulin levels)</td>
<td>ASADI et al., 2012</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>Extract of watercress (hydro-alcoholic)</td>
<td>(Diabetic) rats</td>
<td>• Increased levels of testosterone, LH, FSH, and fast-motility sperm</td>
<td>MOHAMMADI et al., 2017</td>
</tr>
</tbody>
</table>

FSH, follicle stimulating hormone; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; Hb, hemoglobin; LDL, low density lipoprotein; LH, luteinizing hormone; LPO, lipid peroxidation; MCHC, mean corpuscular hemoglobin concentration; MDA, malondialdehyde; NO, nitric oxide; PCO, protein carbonyl; PEITC, phenethyl isothiocyanate; ROS, reactive oxygen species; SOD, superoxide dismutase; TNFα, tumor necrosis factor α, TPA, 12-O-tetradecanoylphorbol-13-acetate.
weeks from April 2017 till November 2017. Cultivation of watercress was established by testing different substrates (soil, soil/sand mixtures, water) and with different concentrations of nutrients (nitrogen and phosphate) and NaCl.

Glucosinolate measurements by HPLC / LC-MS
GLs were analyzed by HPLC according to Boeßtleisch et al. (2017) with modifications. All standard substances were checked for identity by LC-MS. The GL contents in the watercress samples were measured in triplicates.

Human study design and subjects
In a cross-over study, 4 subjects consumed a single dose of 85 g of fresh watercress (along with 50 ml salad dressing, 50 g iceberg lettuce and 50 g cucumber) at breakfast. This amount has been selected since initial human studies also administered 85 g of raw watercress daily (Gill et al., 2007; Fogarty et al., 2013). The diet was compared to a control breakfast (two buns with butter, cheese and 50 g iceberg lettuce). The study was conducted with healthy, untrained human subjects (1 male, 3 females, mean age: 28±6; mean BMI: 22.7±3.5 kg/m²; mean weight: 66±18 kg) who had to complete a high-intensity training session to induce oxidative stress and a pro-inflammatory condition. The subjects were asked to refrain from high-intensity training session to induce oxidative stress and a pro-inflammatory condition. The subjects were asked to refrain from consuming foods rich in polyphenols, vitamin E and vitamin C (anti-inflammatory condition). The subjects were asked to refrain from consuming foods rich in polyphenols, vitamin E and vitamin C (anti-inflammatory condition). The subjects were asked to refrain from consuming foods rich in polyphenols, vitamin E and vitamin C (anti-inflammatory condition). The subjects were asked to refrain from consuming foods rich in polyphenols, vitamin E and vitamin C (anti-inflammatory condition). The subjects were asked to refrain from consuming foods rich in polyphenols, vitamin E and vitamin C (anti-inflammatory condition).

Results

Growth of plants
In 2017, water and plant samples were taken from the outdoor culture system in Poggenhagen (Fig. 1A) every one to two weeks to analyze the nutrient requirements and plant constituents of watercress (data not shown). The biomass production is strongly dependent on the temperature and plants accumulate significantly higher contents of calcium and potassium as the season progresses with peaks reached in mid-summer. To be able to work under controlled conditions, cuttings have been prepared and rooted for propagation in the greenhouse. In the greenhouse, the watercress can be cultivated all year-round with constant biomass growth in 1/2-Hoagland nutrient solution (Fig. 1B). It can also be cultivated on different substrates (mixture of soil and sand or sand), but then the cultivation requires more care. The watercress is relatively salt-tolerant, even at about 120 mM NaCl in the nutrient solution, the plants are vital and show biomass growth (data not shown).

Glucosinolate levels
GLs were analyzed by LC-MS to identify all GLs. Afterwards an HPLC method was applied using detection at 229 nm to be able to analyze many samples in a cost-efficient way. A typical chromatogram is shown in Fig. 2A. The main GL found in watercress is glucosinasturiiin (Fig. 2B). The GL contents differ significantly during the growing season, especially during flowering. Plants grown outside show similar contents of glucosinasturiiin throughout the growing season with elevated levels in flowering plants and plants with developed pods when compared to plants early in the season which are not flowering. Contents of glucobrassicin, neoglucobrassicin and glucoarabishirurinat are lower in plants with developed pods later in the season compared to non-flowering plants early in the season. The contents of the GL 4-methoxyglucobrassicin on the other hand, show an increase in flowering plants with pods compared to plants with flower buds. Overall, the total content of indolic and aliphatic GLs drops in the course of the season, whereas the content of the aromatic GLs elevates slightly (Tab. 5). Flowering had even smaller effects on the GL levels and composition when plants cultivated in the greenhouse (data not shown).

Tab. 4: Health effects of watercress – human studies.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Dosage form</th>
<th>Results/Mechanism(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer and antioxidant</td>
<td>Fresh watercress</td>
<td>Reduced lymphocyte DNA damage</td>
<td>Gill et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altered blood antioxidant status</td>
<td></td>
</tr>
<tr>
<td>Anticancer</td>
<td>Fresh watercress</td>
<td>Genotype dependent increase in GPxs and SOD enzyme activity</td>
<td>Hofmann et al., 2009</td>
</tr>
<tr>
<td>in red blood cells in GSTM1*0</td>
<td></td>
<td>in red blood cells in GSTM1<em>0, but not in GSTM1</em>1</td>
<td></td>
</tr>
<tr>
<td>Anticancer and antioxidant</td>
<td>Fresh watercress</td>
<td>Decrease of exercise induced DNA damage and lipid peroxidation</td>
<td>Fogarty et al., 2013</td>
</tr>
<tr>
<td>Anticancer</td>
<td>PEITC</td>
<td>Inhibition of metabolic activation and lung carcinogenicity</td>
<td>Yuan et al., 2016b</td>
</tr>
</tbody>
</table>

Glucosinolate measurements by HPLC / LC-MS

Glucosinolate levels and composition

GPx, glutathione peroxidase; GSTM1, Glutathion S-Transferase M1; PEITC, phenethyl isothiocyanate.
To analyze the degradation of GLs after cutting the plants, freshly harvested watercress material was stored at 4 °C in the refrigerator in an inflated plastic bag for 1, 2, 3 and 5 d. No significant changes in the gluconasturtiin content occurred (data not shown).

Anti-inflammatory effects of watercress in humans
A trend for increasing concentrations of IL-10, IL-6, and TNFα in LPS-treated whole blood and MDA in plasma was observed 5 min post exercise after the control breakfast suggesting that the exercise protocol was effective in inducing a pro-inflammatory condition and oxidative stress, respectively (Fig. 3). Compared to the control breakfast, the increase of inflammatory cytokines and MDA concentrations 5 min post exercise after the watercress breakfast was lower.

Discussion
Successful cultivation of watercress in the greenhouse
It was demonstrated that watercress can be cultivated all year round in a greenhouse on either sandy substrate or in hydroponic nutrient solution. Therefore, it is not necessary to invent a system with running water. Even without constant aeration watercress produces large amounts of biomass, even at high nutrient or salt concentrations, which is in agreement with the literature (KADDOUR et al., 2013; FERNÁNDEZ et al., 2016). The gain in biomass was strictly temperature-dependent. Therefore, for an all-year-round cultivation a greenhouse would be a prerequisite for controlled hydroponic and aquaponic cultivation.

The content of secondary metabolites changes during the season. Especially flowering has strong effects on the contents of GL. Therefore, the development of a watercress cultivation system avoid-
Hydroponic cultivation and health effects of watercress

Fig. 3: Effect of acute watercress consumption on blood markers of inflammation (IL-10, IL-6, TNFα) and oxidative stress/lipid peroxidation (MDA) after high-intensity training in untrained subjects (n=4). MDA levels were measured in heparin plasma. Inflammatory cytokines were measured in ex vivo LPS-stimulated whole blood cultures.

Tab. 5: Mean concentration of different glucosinolates (GLs) (μmol g DW⁻¹) in watercress in above ground plant material collected at different time points. Plants were growing in an outdoor aquaponic system and harvested to analyze changes in GL contents in the course of the season and developmental stage of the plants. The standard deviation represents the values for three biological replicates.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>4-Methoxy-glucobrassicin</th>
<th>Glucobrassicin</th>
<th>Neo-glucobrassicin</th>
<th>Gluco-arabishirsutain</th>
<th>Gluconasturtiin</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5.2017</td>
<td>0.144 ± 0.060</td>
<td>0.123 ± 0.012</td>
<td>0.063 ± 0.011</td>
<td>0.659 ± 0.117</td>
<td>5.028 ± 0.606</td>
</tr>
<tr>
<td>19.5.2017</td>
<td>0.128 ± 0.007</td>
<td>0.075 ± 0.009</td>
<td>0.04 ± 0.003</td>
<td>0.457 ± 0.111</td>
<td>5.427 ± 0.232</td>
</tr>
<tr>
<td>26.5.2017*</td>
<td>0.122 ± 0.018</td>
<td>0.109 ± 0.026</td>
<td>0.041 ± 0.110</td>
<td>0.390 ± 0.127</td>
<td>4.882 ± 0.782</td>
</tr>
<tr>
<td>09.6.2017**</td>
<td>0.176 ± 0.022</td>
<td>0.09 ± 0.016</td>
<td>0.041 ± 0.017</td>
<td>0.369 ± 0.106</td>
<td>6.017 ± 0.310</td>
</tr>
<tr>
<td>16.6.2017</td>
<td>0.187 ± 0.035</td>
<td>0.072 ± 0.010</td>
<td>0.043 ± 0.027</td>
<td>0.298 ± 0.146</td>
<td>6.418 ± 0.229</td>
</tr>
<tr>
<td>23.6.2017***</td>
<td>0.167 ± 0.079</td>
<td>0.038 ± 0.028</td>
<td>0.031 ± 0.011</td>
<td>0.219 ± 0.075</td>
<td>5.101 ± 1.691</td>
</tr>
</tbody>
</table>

*first flowers, **many flowers, ***no flowers anymore.

Anti-inflammatory effects of watercress in humans – a new approach

Anti-inflammatory effects of watercress have not yet been investigated in humans. Isothiocyanates in general have been shown to possess anti-inflammatory properties through the regulation of cytokines of the TNF family primarily via activation of the NF-κB pathway (Heiss et al., 2001; Wierinckx et al., 2005; Dey et al., 2006; Karmakar et al., 2006). Also, studies with knockout mice showed that the nuclear factor erythroid 2-related factor 2 (Nrf2) plays an important role in the anti-inflammatory and antioxidative effects of PEITC (Boyanapalli et al., 2014).

Although, the differences of IL-10, IL-6, TNFα and MDA concentrations between control and watercress were not statistically significant due to high interindividual differences and a low case-number, the results of the present pilot study can be interpreted as a trend towards an anti-inflammatory and antioxidant effect of fresh watercress even at a single dose. Measurable effects after a single dose of watercress were also shown by Fogarty et al. (2013). In particular, the applied model for the induction of oxidative stress and inflammation in combination with cytokine analysis in whole blood cultures after ex vivo immune cell stimulation appears applicable to the relevant question. To consolidate these preliminary results, we plan to apply the outlined study protocol with a larger sample size over a longer intervention time in future studies. In addition, studies with patients suffering from diseases associated/accompanied with increased oxidative stress and inflammation (e.g. asthma) are planned. In these studies, additional biomarkers are necessary to examine the beneficial effects of watercress on human health. However, long-term studies with fresh watercress are difficult to realize due to its sharp taste,
gastric discomfort causing effects (two subjects complained about slight gastric discomfort after the watercress consumption), limited shelf life, and elaborate logistics in the daily distribution.

Conclusion/Outlook
As demonstrated, watercress can be successfully cultivated all year round in the greenhouse without a reduction in its valuable contents. To optimize the utilization of nutrients, the hydroponic culture will be combined with the cultivation of fish in one greenhouse in the near future. To circumvent the problem of limited shelf life other methods will be developed to provide humans with the valuable metabolites of watercress. The human pilot study indicates that fresh watercress has positive effects on oxidative stress and inflammatory markers under induced pro-oxidative and pro-inflammatory conditions. Future long-term intervention studies with larger collectives must be carried out to confirm these results. In the ongoing project we aim to develop watercress dosage forms to overcome these obstacles. Processing technologies like gentle freeze-drying, grinding, subsequent encapsulation and gastric juice resistant coatings appear proper to ensure high tolerability, preservation of valuable ingredients (primarily glucosinolates and myrosinase), high bioavailability and compliance in long-term intervention studies. In future clinical studies with watercress extracts, the dose and duration must be carefully considered. A recent study observed an effect of PEITC in high concentrations on accumulation of reactive oxygen species and cytoskeletal changes, resulting as a consequence of cytotoxicity (Dayalan Naidu et al., 2018).

Authors’ contributions
JPS, AH and JP conceived and designed the experiments. JPS, PW, MRL and JH performed the experiments. JPS, AH, TG and JP analyzed the data and prepared the manuscript. All authors read and approved the manuscript.

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Hadjzadeh, M.A.R., Rajaei, Z., Moradi, R., Ghorbani, A., 2015: Effects of Hydroalcoholic extract of watercress (Nasturtium officinale) leaves on serum glucose and lipid levels in diabetic rats. Indian J. Physiol,
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