100 YEARS
Journal of Applied Botany

Journal of Applied Botany and Food Quality 92, 232 - 239 (2019), DOI:10.5073/JABFQ.2019.092.032

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Watercress – cultivation methods and health effects

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(Submitted: May 18, 2019; Accepted: July 20, 2019)

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-Szczykutowicz 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 dithiocarbamates (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.

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

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

Effect	Dosage form	Cell line/ experimental model	Results/Mechanism(s)	Reference
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	Boyd et al., 2006
Anticancer	PEITC	Biliary tract cancer cells	• Reduction in cisplatin resistance • Increased rate of apoptosis of cancer cells • Inhibited xenograft tumor growth	Li et al., 2016
Anticancer	Extract of watercress	Human colon cancer cells (HT29 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 cardio-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-picrylhydrazyl, 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	Spínola et al., 2017
Antioxidant	Extract of watercress (aqueous and ethanolic)	Direct measurement of antioxidant capacity of watercress extract	• Improved total antioxidant activity, reducing power, DPPH* radicals and superoxide anion radicals scavenging activities	Ozen, 2009
Antioxidant and anticancer	Extract of watercress & PEITC	Human PBMC	• Increased gene expression of detoxification enzymes (GP×1 and SOD2) • Increased SOD2 activity	Hofmann 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; GP×1, glutathione peroxidase 1; IL-10, Interleukin 10; NO, nitric oxide; PEITC, phenethyl isothiocyanate; SOD, superoxide dismutase; TNFα, tumor necrosis factor α.

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. 3: Health effects of watercress – animal studies.

Effect	Dosage form	Species	Results/Mechanism(s)	Reference
Anticancer	Aqueous solution of watercress	Swiss mice	• Suppression of Ehrlich tumor growth	DE SOUZA et al., 2016
Anti-inflammatory	Extract of watercress	Rats	• Inhibition of carrageenan-induced paw edema • Activity against formalin-evoked paw edema • Decreased swelling and tissue damage induced by carrageenan or TPA	SADEGHI et al., 2014
Antioxidative, hypolipemic and cardioprotective	Extract of watercress	(Hypercholesterolaemic) rats	• Decrease of hepatic MDA, GR and GPx activities • Reduced total cholesterol, triglycerides and low-density lipoprotein • Increased levels of blood high-density lipoprotein cholesterol	YAZDANPARAST et al., 2008
Antioxidant and anti-inflammatory	Extract of watercress	Rats	• Protection against increase in ROS, GSH, LPO and PCO in gentamicin-induced nephrotoxicity • Protection against increase in NO and TNF**α** in gentamicin-induced nephrotoxicity	SHAHANI et al., 2017
Antioxidant and antidiabetic	Extract of watercress	(Diabetic) rats	• Improvement of antioxidant status: SOD, GR, GPx, MDA in plasma and different tissues, total antioxidant status • Hypoglycemic effect of aqueous watercress extract was 76.6 % higher than that of insulin; glucose levels were normalized on the third week up to the eighth week	FENTON-NAVARRO et al., 2018
Antidiabetic	Different extracts of watercress (ethyl acetate, methanol and aqueous)	(Diabetic) rats	• Decrease of blood glucose after 1 week and 2 months	HOSEINI et al., 2009
Antidiabetic and hypolipidemic	Extract of watercress (hydro-alcoholic)	(Diabetic) rats	• Decrease of serum glucose, total cholesterol and LDL-cholesterol	HADJZADEH et al., 2015
Antioxidant	Extract of watercress (hydro-alcoholic)	Rats	• Attenuation of Vancomycin-induced nephrotoxicity • MDA levels decreased compared to control	KARAMI et al., 2018
Antioxidant	Watercress juice	Mice	• Enhancement of superoxide dismutase activity in erythrocytes • Improved glutathione balance • Diminished lipid oxidation in all matrices	CASANOVA et al., 2017
Antioxidant	Extract of watercress (ethanolic)	Rats	• Decreased lipid peroxidation in liver, brain and kidney	OZEN, 2009
Antioxidant	Watercress oil	Rabbits	• Improved SOD activity and GSH concentrations	ALAGAWANY et al., 2018
Immuno-modulating	Extract of watercress	Rainbow trout	• Enhancement of hematological and immunological parameters (including Hb and MCHC, lysozyme and complement activities, total protein and globulin levels)	ASADI et al., 2012
Reproductive system	Extract of watercress (hydro-alcoholic)	(Diabetic) rats	• Increased levels of testosterone, LH, FSH, and fast-motility sperm	MOHAMMADI et al., 2017

FSH, follicle stimulating hormone; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; Hb, hemoglobin; LDL, low density lipoprotein; LH, 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.

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

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

Effect	Dosage form	Results/Mechanism(s)	Reference
Anticancer and antioxidant	Fresh watercress	• Reduced lymphocyte DNA damage • Altered blood antioxidant status	GILL et al., 2007
Anticancer	Fresh watercress	• Genotype dependent increase in GPx and SOD enzyme activity in red blood cells in GSTM1*0, but not in GSTM1*1	HOFMANN et al., 2009
Anticancer and antioxidant	Fresh watercress	• Decrease of exercise induced DNA damage and lipid peroxidation	FOGARTY et al., 2013
Anticancer	PEITC	• Inhibition of metabolic activation and lung carcinogenicity of tobacco	YUAN et al., 2016b

GPx, glutathione peroxidase; GSTM1, Glutathion S-Transferase M1; PEITC, phenethyl isothiocyanate.

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 BOESTFLEISCH 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/m^2; 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 consuming foods rich in polyphenols, vitamin E and vitamin C (especially berries, grapes, nuts), 7 days before the start of the study. On each of the 2 study days, fasting blood was taken from the subjects in the morning (baseline), followed by the consumption of the test breakfast (watercress or control). 120 minutes after breakfast, the subjects completed a 30-minute continuous running based endurance workout combined with bodyweight strength-endurance exercises (burpees, push-ups, squats and sit-ups) at 80% of their maximal heart rate (HRmax). Participants were instructed to perform 10 reps of each exercise in between replicated 400 m track runs. Throughout the workout, the HR was continuously recorded using a Polar A300 Fitness and Activity Tracker with a H7 Bluetooth heart rate sensor (Polar Electro Oy, Kempele, Finland). Five minutes after the training session, the second blood draw was taken (5 min post-exercise). The subjects were allowed to drink water ad libitum.

Concentrations of inflammatory cytokines and MDA were determined at baseline and 5 min post-exercise. Blood samples were obtained by venipuncture of an arm vein using Multifly needles (Sarstedt, Nümbrecht, Germany) into heparin plasma monovettes (Sarstedt, Nümbrecht, Germany). MDA was analyzed in heparin plasma using the TBARS Assay Kit from Cayman chemical (Biomol, Hamburg, Germany) according to the manufactures instructions. IL-10, IL-6 and TNFα were analyzed in whole blood cultures after *ex vivo* immune cell stimulation via lipopolysaccharide (LPS). Briefly, heparinized blood samples were diluted 1:4 with RPMI 1640 including HEPES and L-glutamine (Sigma-Aldrich, Hamburg, Germany) and added antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin, Sigma-Aldrich, Hamburg, Germany). The blood was seeded into 12-well microtiter plates and stimulated with 10 ng/ml (final concentration) of LPS (Sigma-Aldrich, Hamburg, Germany) or medium alone in duplicates. Blood was incubated at 37 °C for 24 h. After incubation, IL-10, IL-6 and TNFα were simultaneously quantified in whole blood culture supernatant using a Bio-Plex Multiplex Immunoassay including a Bio-Plex MAGPIX™ multiplex reader.

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 gluconasturtiin (Fig. 2B). The GL contents differ significantly during the growing season, especially during flowering. Plants grown outside show similar contents of gluconasturtiin 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 glucoarabishirsutain 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).

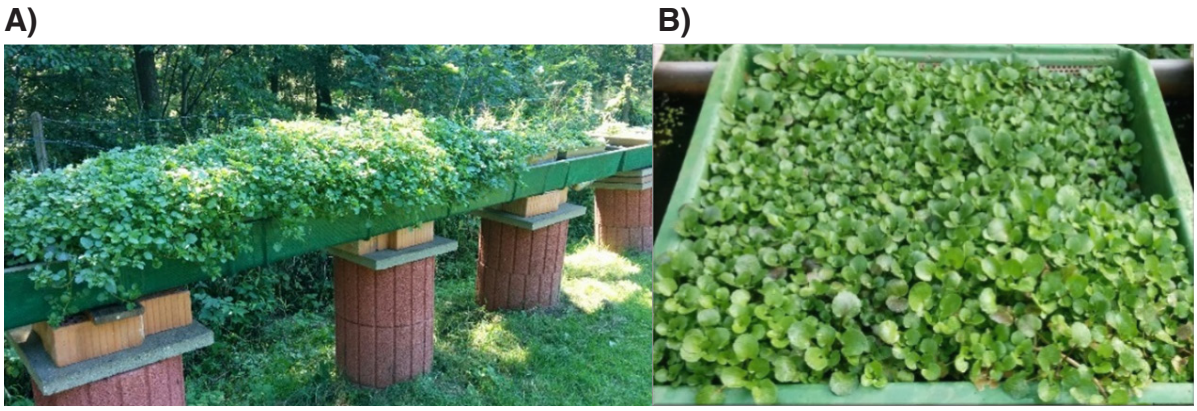

Fig. 1: Cultivation of watercress in A) Poggenhagen in the field and in the B) greenhouse.

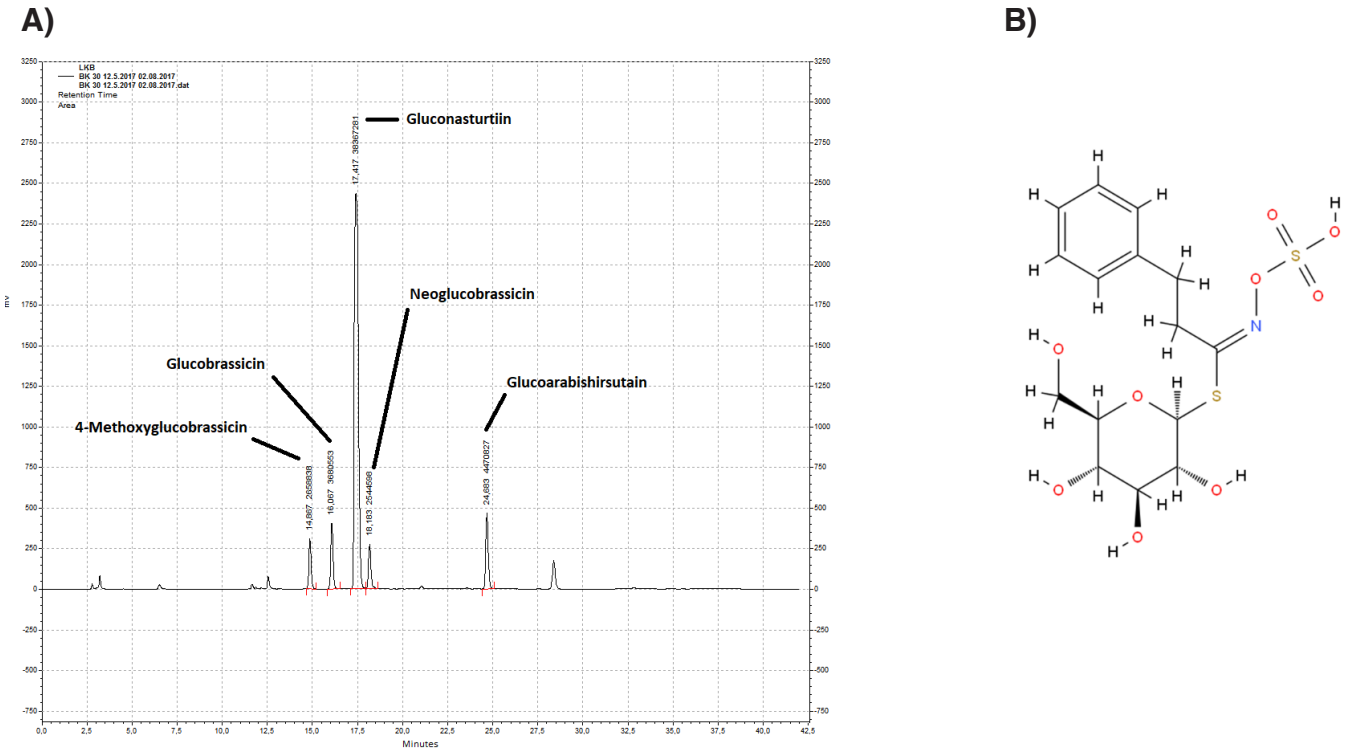

Fig. 2: A) HPLC chromatogram of a watercress extract. B) Gluconasturtiin (phenethyl glucosinolate, Chem Spider ID 7827641) is the most abundant glucosinolate in the eponymous watercress *Nasturtium officinale*.

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; FERNÀNDEZ 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-

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.

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

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

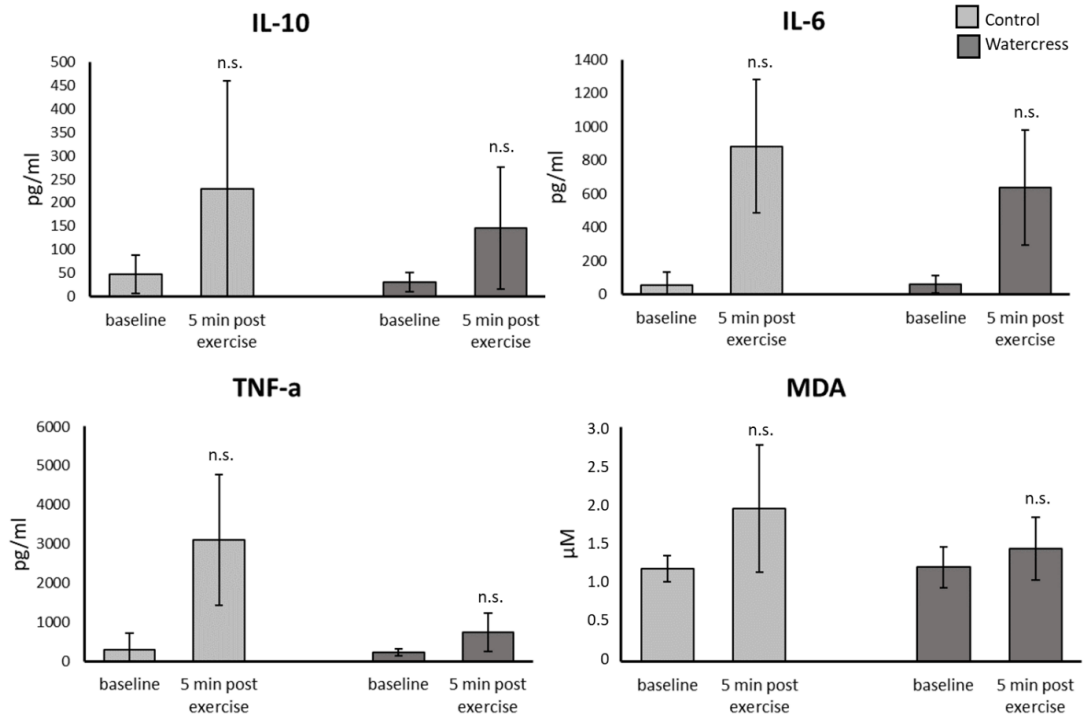

Fig. 3: Effect of acute watercress consumption on blood markers of inflammation (IL-10, IL-6, TNFα) and oxidative stress/lipid peroxidation (MDA) after high-intensity training in untrained subjects (n=4). MDA levels were measured in heparin plasma. Inflammatory cytokines were measured in *ex vivo* LPS-stimulated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

ing flowering at all would be preferable. Temperature probably also contributes to changes in GL content and pattern, since contents seem to be influenced not only by daytime temperature, but also by the difference between daytime and nighttime temperature (Engelen-Eigels et al., 2006).

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 gluconasturtiin 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.

Acknowledgments

We would like to thank Stefan Göckemeyer, Poggenhagen, who drew our attention on this traditional vegetable and supported us with plants and advices on the outdoor cultivation of watercress. The authors acknowledge the financial support by the Faculty of Natural Sciences, Leibniz University Hannover, within the research initiative Soil - Plant - Human Interactions.

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