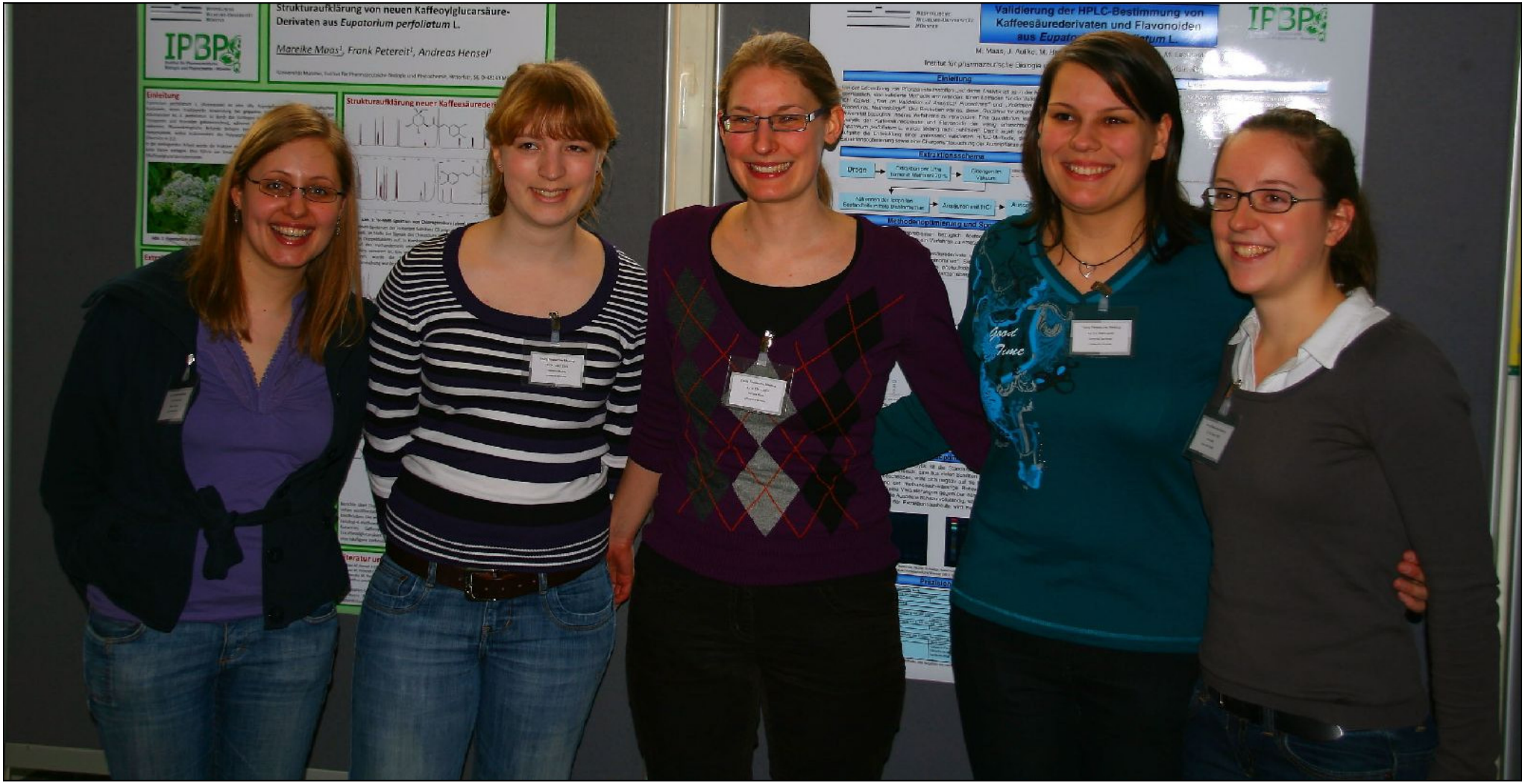
Strukturaufklärung von neuen Kaffeoylglucarsäure-Derivaten aus Eupatorium perfoliatum L.
Mareike Maas[1], Frank Petereit[1], Andreas Hensel[1]
IPBP
Einleitung
Validierung der HPLC-Bestimmung von Kaffeesäurederivaten und Flavonoiden
Extraktionsschema
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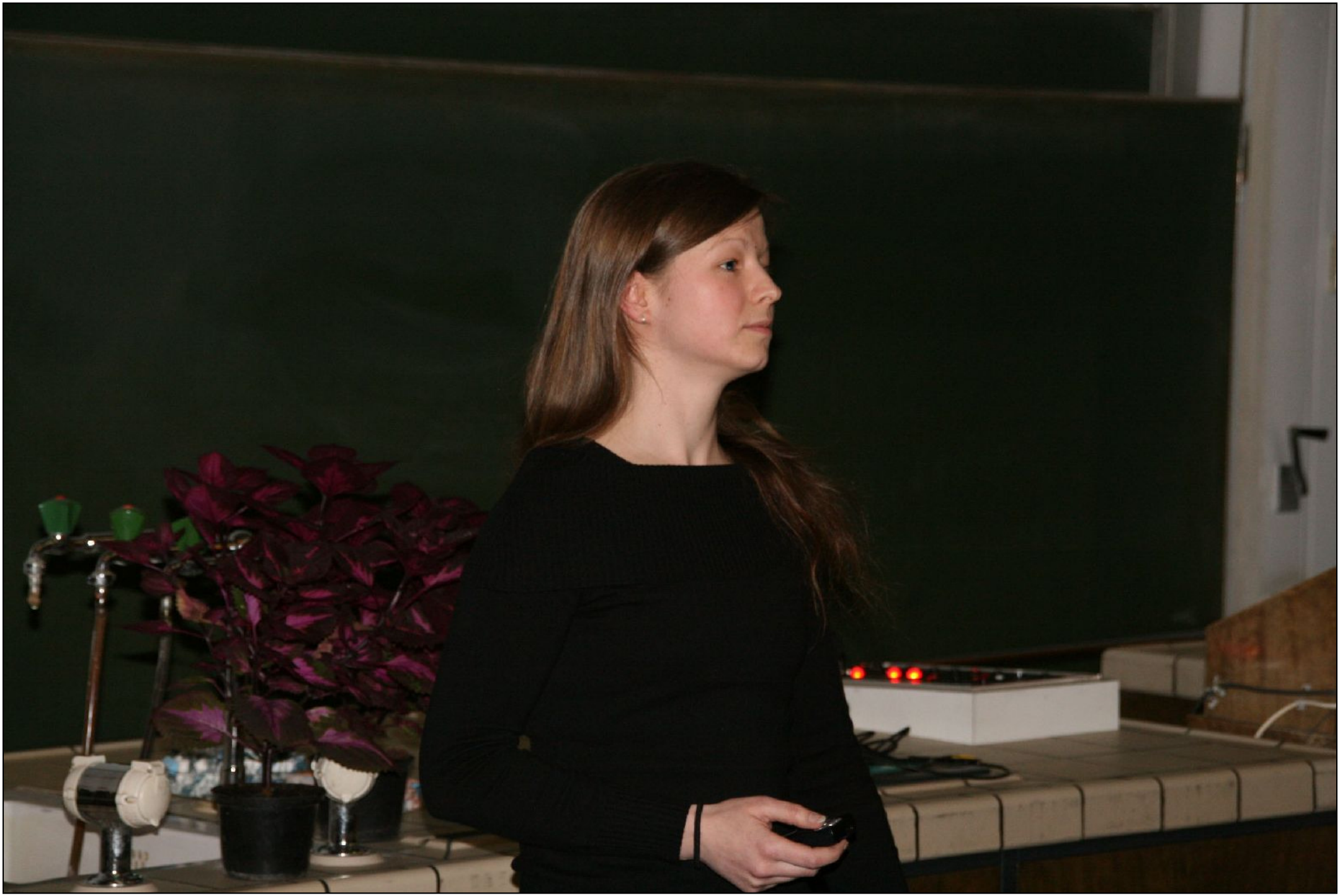
Good Time


IP3P
Einleitung


Young Researcher Meeting
Phytopharmaka in der aktuellen Forschung
Westfälische Wilhelms-Universität Münster
13. und 14. März 2009
KLOSTERFRAU
HEALTHCARE GROUP
PROPHYTO




IP3P
Hemmung der TNF-α-Sekretion
IP3P


NESCAFÉ


Growth inhibiting activities of Dipsacus sylvestris HUDS. against
Borrelia burgdorferi sensu stricto (Bbss) in vitro
Notausgang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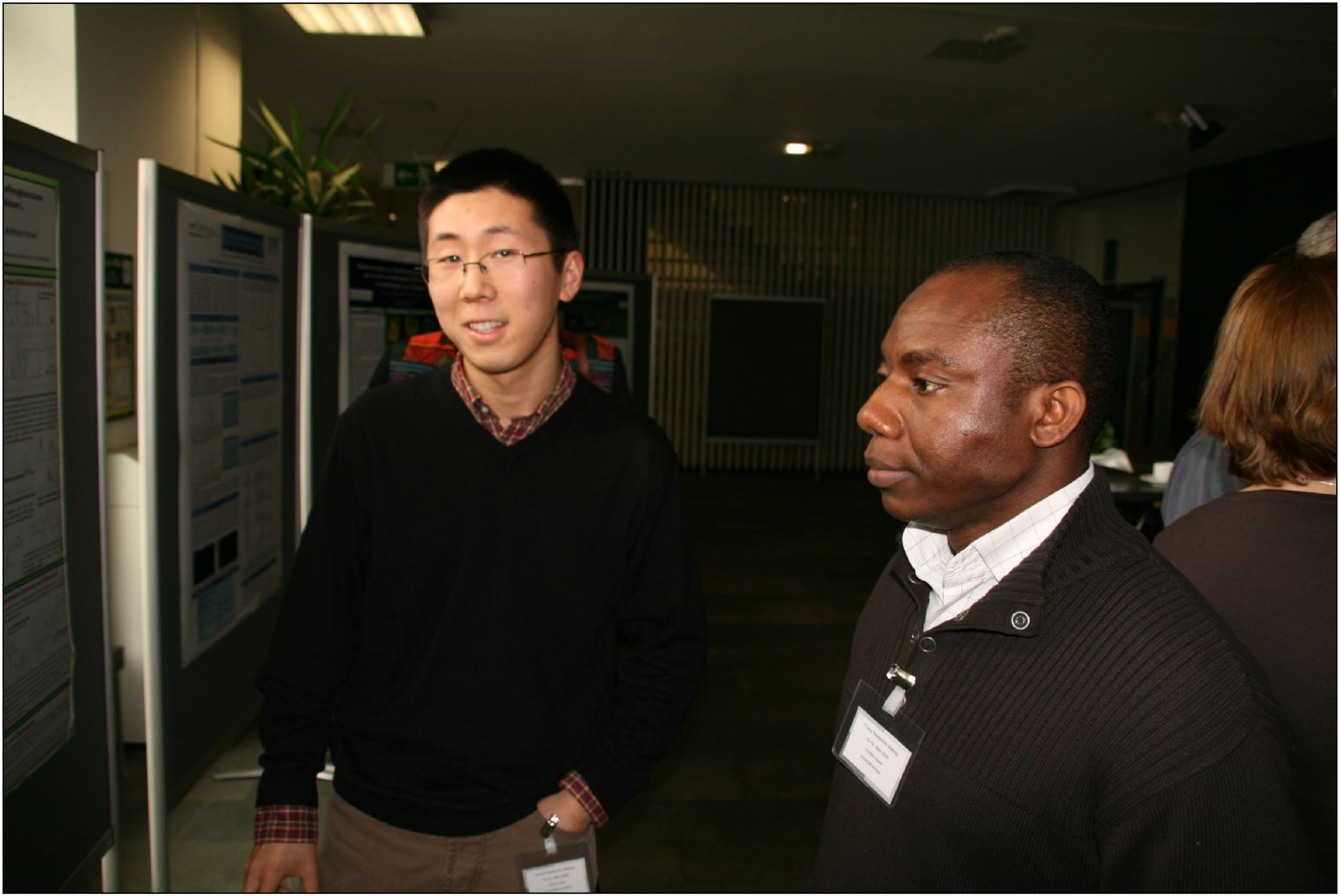

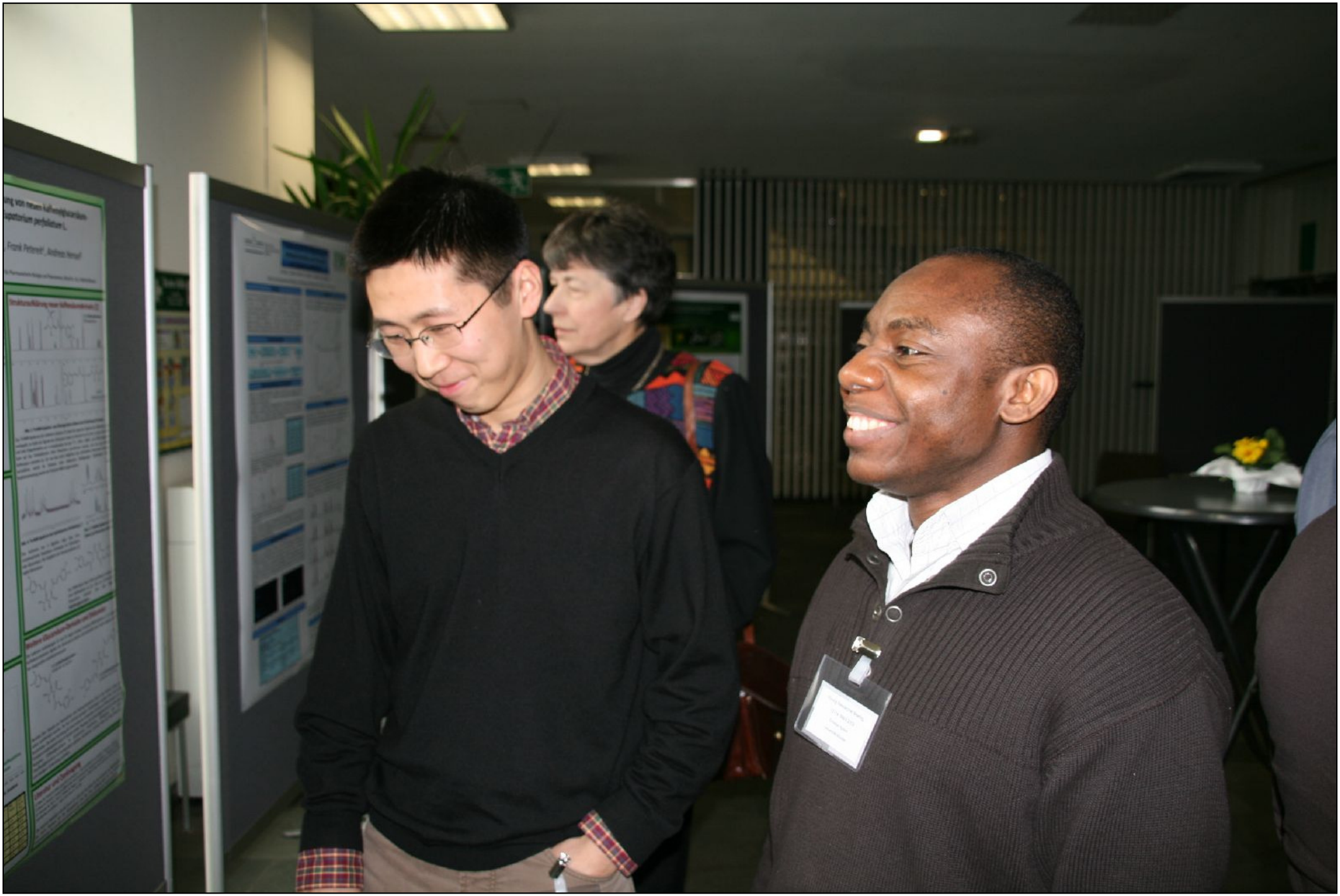


HPLC - fingerprint
Leonurus cardiac
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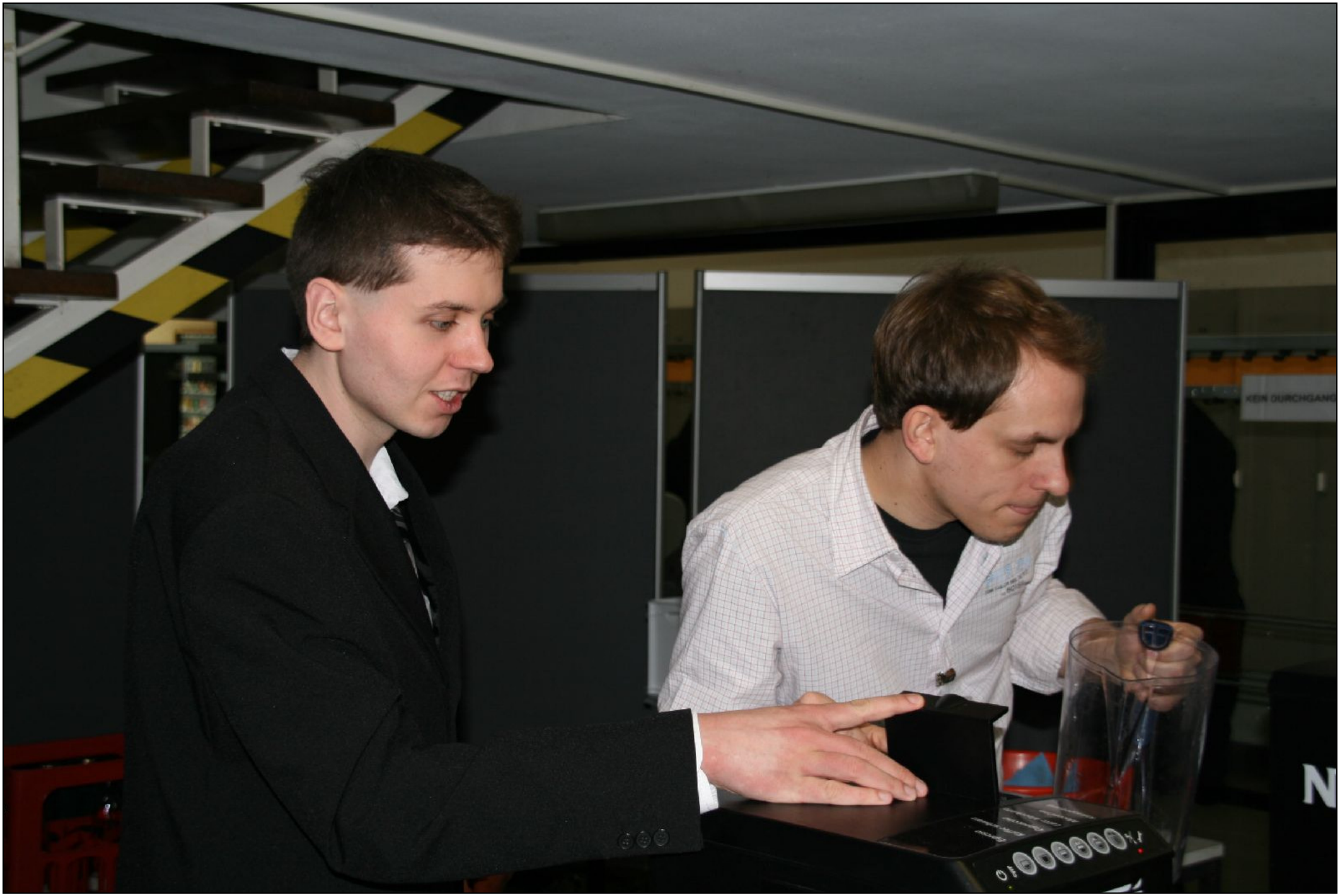
KEIN DURCHGANG


Young Researcher Meeting
11./14. März 2009
Universität Leipzig


selected Ghanaian plants on
Trocyfes
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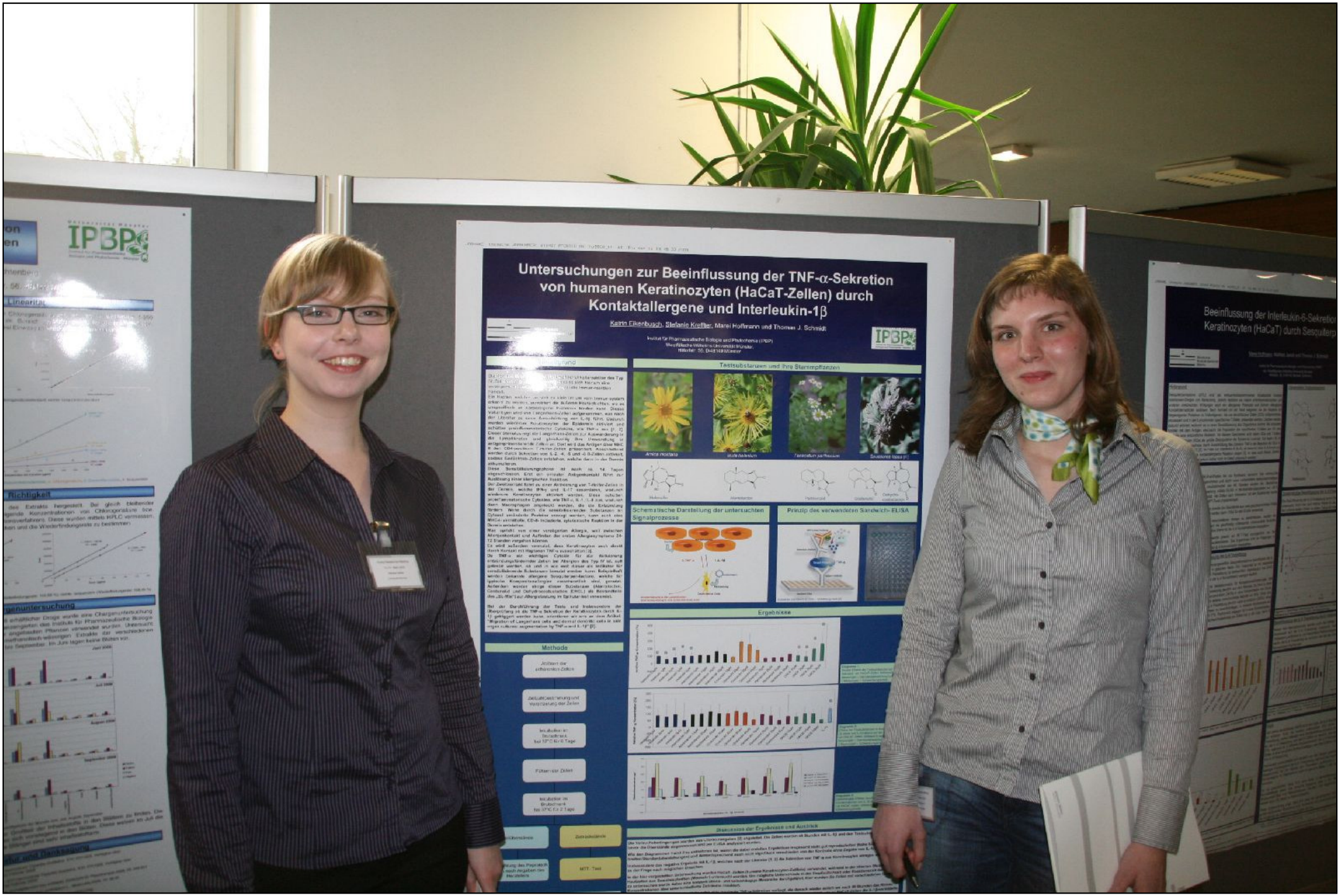
wissen leben
WWU Münster
Mathias Jacob


Untersuchungen zur Beeinflussung der TNF-α-Sekretion von humanen Keratinozyten (HaCaT-Zellen) durch Kontaktallergene und Interleukin-1β
Kathrin Eikenbusch, Stefanie Kreffter, Marei Hoffmann und Thomas J. Schmidt
Testsubstanzen und ihre Stammpflanzen
Schematische Darstellung der untersuchten Signalprozesse
Prinzip des verwendeten Sandwich-ELISA
Ergebnisse
Methode
Diskussion der Ergebnisse und Ausblick
Beeinflussung der Interleukin-6-Sekretion
Keratinozyten (HaCaT) durch Sesquiterp

Phytochemical screening and effect of selected Ghanaian plants on viability of skin keratinocytes

C. Agyare, M. Lechtenberg, A. Deters, A. Hensel

Institute for Pharmaceutical Biology and Phytochemistry, Corrensstrasse 48, 48149 Muenster, Germany

Young Researcher Meeting, Muenster, March 2009

Introduction

Wounds have a tremendous impact on the healthcare economy [illegible] represent a major health burden and drain on healthcare resources [illegible] countries (JanBen, 2006). In Ghana and Africa, it estimated that 70% [illegible] treated by traditional healers and herbal practitioners or rely on [illegible] their health needs (Nyika, 2007; Farnsworth, 1988). People in [illegible] medicine for their health needs including management of wounds [illegible] cost of orthodox medicines, inadequate health facilities and [illegible] coupled with lack of training of health workers on skin disorders [illegible] et al., 2006), easy availability, affordability of herbal medicines [illegible]. One of the methods in which drugs are discovered is through [illegible] has been proven to be reliable. Medicinal plants have [illegible] management of skin disorders and as wound healing agents [illegible] 2007). We conducted an ethnopharmacological survey in [illegible] district in Ghana and identified 103 plants belonging to 45 families as wound healing agents (Agyare et al., 2007). Based on the frequency of use and [illegible] on the identified plants, a few selected plants have been investigated [illegible] healing principles or agents.

Aims

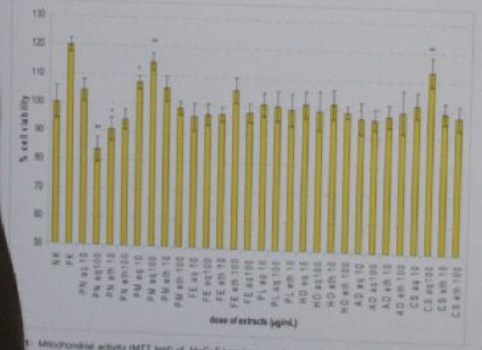
- To identify some of the secondary metabolites in seven selected Ghanaian plants through phytochemical screening.
- To determine the effect of both aqueous and ethanolic extracts of these plants on the proliferation of HaCaT keratinocytes cells by means of cell viability (MTT-test).

Results and discussion

Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, carbohydrates (mainly glucose and saccharose) and tannins in selected plant extracts (Table 1). The aqueous extract of the dried root of *A. difformis* contains starch. Hydrolyzed polysaccharides were mainly arabinose and galactose, indicating the frequent occurrence of arabino-galactan (Table 2). Both glucuronic and galacturonic acids were present in *P. muellerianus* and *P. lappacea* extracts and glucuronic acid was present in *P. nigrencens* and *A. difformis*. Galacturonic acid was found in *F. exasperata* and *H. opposita* extracts.

The aqueous extracts of *P. muellerianus* and *C. smeathmanni* significantly increased mitochondrial activity of the HaCaT cells between 7-15% compared to untreated controls (Fig. 1). This indicates that both plant extracts increased cell viability of HaCaT keratinocytes. *P. muellerianus* aqueous extracts showed dose-dependent activity in the range from 10 to 100 µg/mL whilies the 200 µg/mL treated group was less stimulated (Fig. 2). Both aqueous and ethanolic extracts of *P. nigrencens* were found to be cytotoxic at a concentration of 100 µg/mL.

Further studies would be conducted to find out effects of these extracts on the proliferation of keratinocytes, skin fibroblasts, collagen production, cytotoxicity, antimicrobial and antioxidant activities to ascertain the wound healing properties of the bioactive isolates/extracts. Bioactivity-guided isolation and characterization of the bioactive principles/isolates will be done.



Mitochondrial activity (MTT test) of HaCaT keratinocytes incubated with 10 and 100 µg/mL of [illegible] extracts (aq) and ethanolic extracts (eth) over 48 h incubation period. Bars represent [illegible] deviation (SD) with n=10 replicates from 3 independent experiments with * p= 0.05 and [illegible] 0.01 compared (T-test) to the untreated control group. NK: negative control, untreated, [illegible] control (1 % FCS).

Methodology

Preparation of plant extracts

Aqueous extracts: 10 g of the powdered plant material were added to 100 mL of distilled water and heated at 90°C for 15 min., centrifuged, the supernatant concentrated and lyophilized.

Ethanolic extracts: 10 g of the powdered plant material was added to 100 mL ethanol/water (60:40 v/v) and heated at 70°C for 30 min, centrifuged, the supernatant concentrated and lyophilized.

Phytochemical screenings were conducted on dried leaves of *Parquetina nigrencens* (Afzel.) Bullock (Asclepiadaceae), *Phyllanthus muellerianus* Kuntze (Euphorbiaceae), *Ficus exasperata* Vahl. (Moraceae), *Pupalia lappacea* (Linn.) Juss. (Amaranthaceae), *Hoslundia opposita* Vahl.(Labiatae), *Combretum smeathmanni* G. Don. (Combretaceae), and dried root of *Anchomanes difformis* (Bl.) Engl.(Araceae) for flavonoids, alkaloids, carbohydrates, saponins (Wagner & Bladt., 1996; Harborne, 1984) and quantity of tannins was determined according to the method of Glasl (1983). Polysaccharides were obtained after precipitation of aqueous extracts in ethanol with a final concentration of 80%, concentrated and lyophilized. Polysaccharides were hydrolyzed with trifluoroacetic acid at 121°C for 60 min. TLC was performed on silica gel F_{254} plates using acetonitrile-water (80:20 v/v) as mobile phase (developed 3 times) and monosaccharides were detected with Thymol-sulphuric acid reagent after heating at 120°C for 5 min. The monosaccharides and uronic acids were additionally quantified by HPAEC-PAD.

Effects of extracts on HaCaT keratinocytes cells

Both aqueous and ethanolic extracts (10 and 100 µg/mL) were dissolved in a defined keratinocyte medium (MCDB 153 complete medium) and were added to 96-well cell plates with HaCaT keratinocytes cells (5 x 10^4cells/well). The cells were cultured at 5% CO_2 in 95% humidified atmosphere at 35°C. After 48 h of incubation, cell viability was measured by MTT Test (Deters et al., 2005). Dose-dependent activity was determined for the extracts which showed significant activity or influence on the HaCaT cells.

Table 1: Results of phytochemical screening of the plants samples

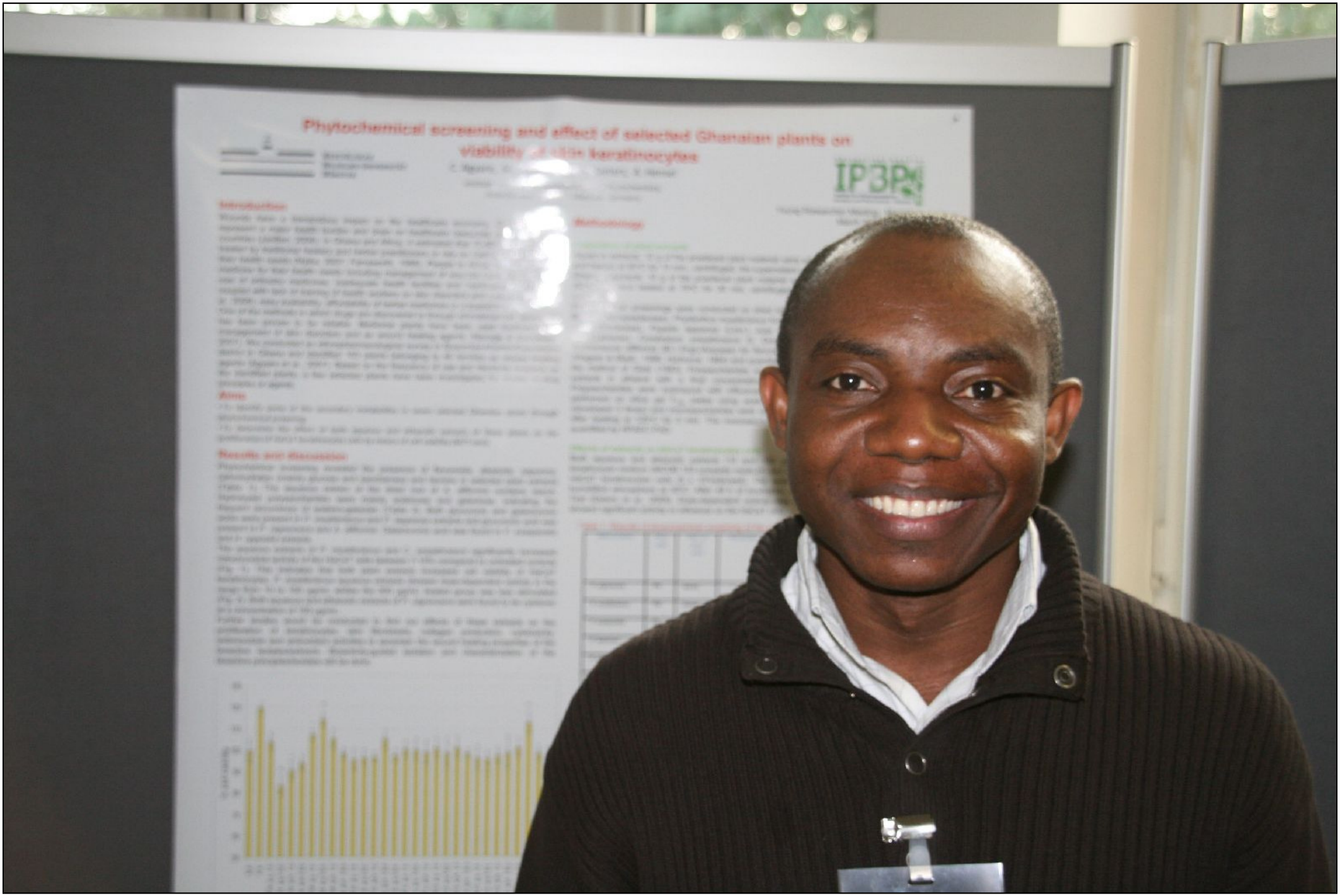
Name of plant	Part used	Part of plant used	Flavonoids	Saponins	Alkaloids	Carbohydrates	Tannins (%w/w)
P. nigrencens	PN	leaves	+	+++	-	+	1.1
P. muellerianus	PM	leaves	+	+++	+	+	13.7
F. exasperata	FE	leaves	+	+++	-	+	1.3
P. lappacea	PL	leaves	+	+++	-	+	1.5
H. opposita	HO	leaves	+	++	-	+	1.8
C. smeathmanni	CS	leaves	+	+++	-	+	16.5
A. difformis	AD	roots	-	+++	-	+	0.04

Table 2: Yield and composition of polysaccharides from the aqueous plant extracts from HPAEC-PAD

Plant code	PN	PM	FE	PL	HO	AD	CS
Yield of polysaccharides (% w/w)	3.4	3.8	5.2	5.5	0.8	0.8	10.0
Monosaccharides (%)							
Arabinose	30.4	14.5	1.8	17.5	7.8	2.3	15.4
Xylose	-	-	-	4.7	5.7	4.0	-
Galactose	32.8	19.2	28.2	6.5	-	-	5.0
Glucose	25.8	44.7	32.8	47.3	68.8	27.9	15.8
Mannose	5.2	5.5	8.6	5.1	-	1.6	-
Rhamnose	3.6	6.2	25.8	5.8	9.5	12.6	2.1
Fructose	1.7	4.1	0.8	7.8	10.3	42.7	1.6
Ribose	1.1	1.8	1.7	1.1	14.0	14.3	11.4

Acknowledgements

Authors are grateful to German Academic Exchange Service (DAAD) for the scholarship offered Mr. C. Agyare for his PhD program and Mr. Ntim Gyakari, Botanist, Forestry Services and Dr. Alex Asase, Department of Botany and Ghana Herbarium, University of Ghana, Ghana, for the identification of the plants and to traditional healers in Bosomtwi-Atwima-Kwanwoma district, Ghana.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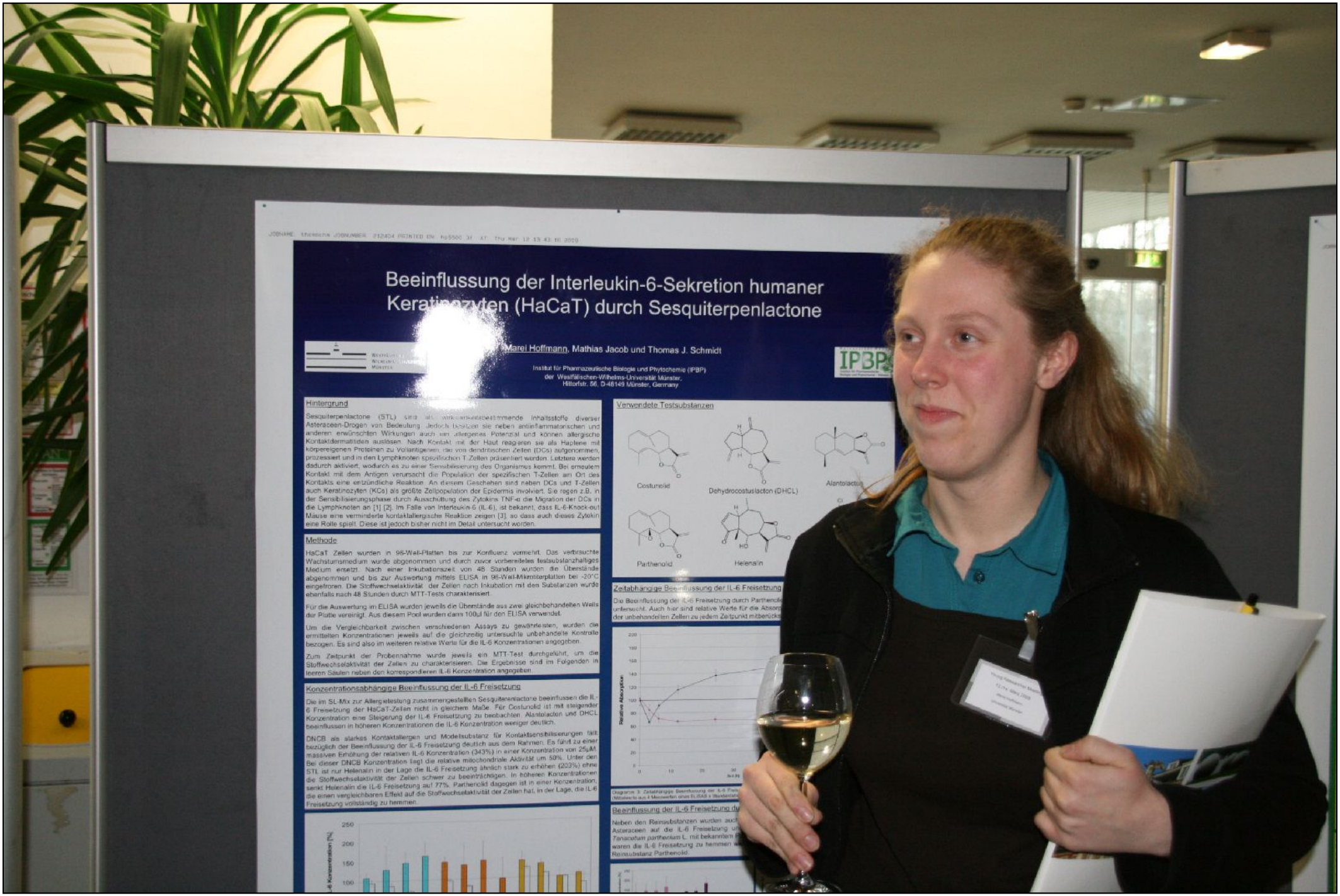
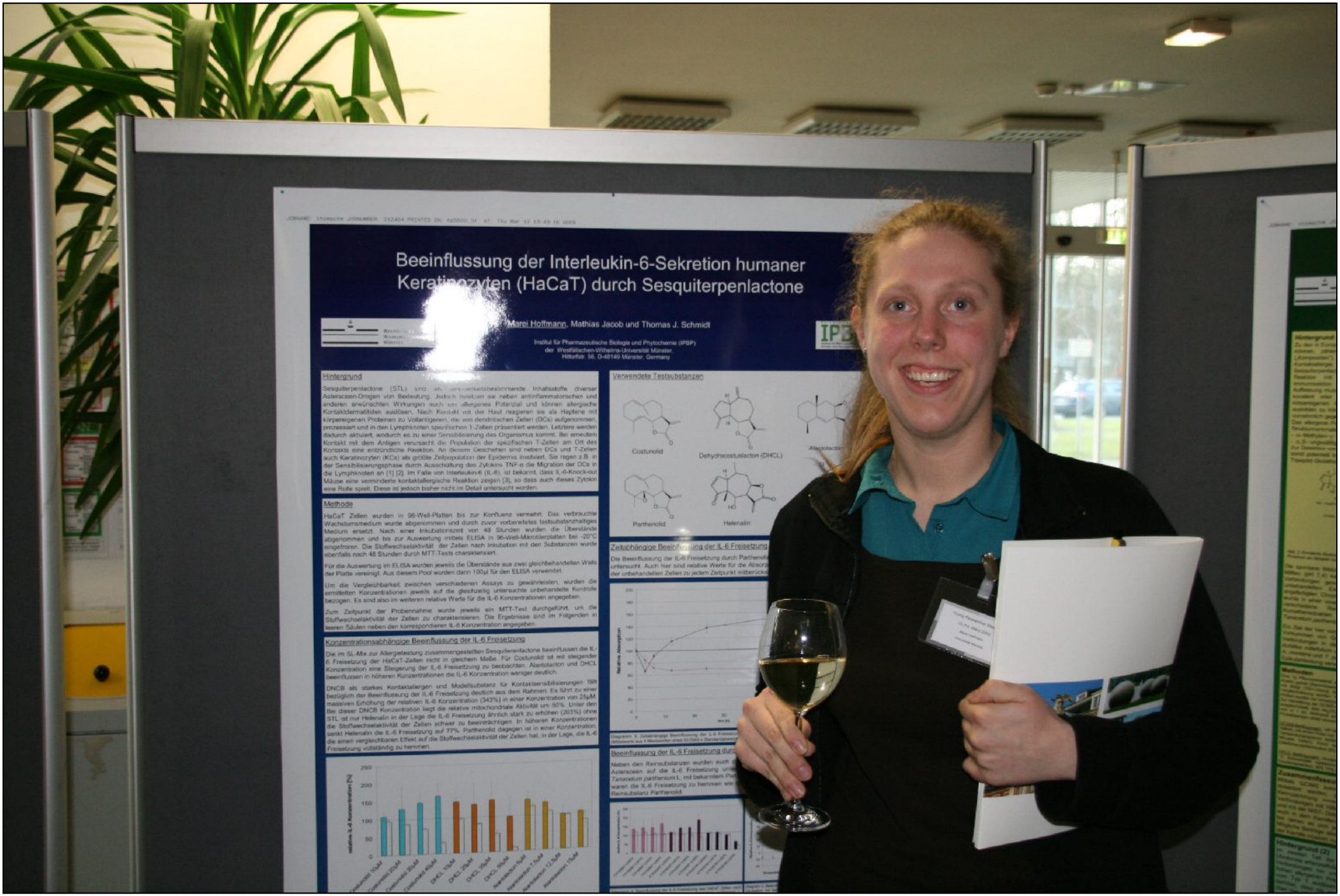

Beeinflussung der Interleukin-6-Sekretion humaner Keratinozyten (HaCaT) durch Sesquiterpenlactone
Marei Hoffmann, Mathias Jacob und Thomas J. Schmidt
Institut für Pharmazeutische Biologie und Phytochemie (IPBP)
der Westfälischen-Wilhelms-Universität Münster,
Hittorfstr. 56, D-48149 Münster, Germany
Hintergrund
Methode
Konzentrationsabhängige Beeinflussung der IL-6 Freisetzung
Verwendete Testsubstanzen
Costunolid
Dehydrocostuslacton (DHCL)
Alantolacton
Parthenolid
Helenalin
Zeitabhängige Beeinflussung der IL-6 Freisetzung
Beeinflussung der IL-6 Freisetzung du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IPB
Hintergrund
Methode
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Costunolid
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Parthenolid
Helenalin
Zeitabhängige Beeinflussung der IL-6 Freisetzung
Konzentrationsabhängige Beeinflussung der IL-6 Freisetzung
relative IL-6 Konzentration [%]
Costunolid 10µM
Costunolid 20µM
Costunolid 30µM
Costunolid 40µM
DHCL 15µM
DHCL 25µM
DHCL 35µM
DHCL 55µM
Alantolacton 5µM
Alantolacton 7,5µM
Alantolacton 12,5µM
Alantolacton 15µM

Rhododendron ferrugineum L. –
Phytochemische und funktionelle Untersuchungen einer traditionellen Arzneipflanze

IPBP

A. Louis[1] und A. Hensel[1]

[1]Universität Münster, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstr. 56, 48149 Münster

Einleitung

Rhododendron ferrugineum L., die rostrote Alpenrose (Ericaceae) findet ihre Verbreitung in der gesamten Alpenkette von den Seealpen bis nach Niederösterreich, den Pyrenäen, dem Appenin und dem südkroatischen Gebirge. Sie ist auf einer Höhe von 1500 bis 2800 m zu finden. Als traditionelle Arzneipflanze fand *R. ferrugineum* L. Anwendung bei rheumatischen Beschwerden, Gicht und Steinbildung. Aufgrund der unklaren und unvollständigen Datenlage zur phytochemischen Charakterisierung der Droge und Fallberichten zur Vergiftung von Weidevieh veröffentlichte die Kommission E 1990 eine Negativmonographie für Rhododendri ferruginei folium [1, 2].

Ziel der vorliegenden Arbeit war es, die wichtigsten Inhaltsstoffe der Blattdroge zu charakterisieren und ihre Funktionalität an einem Hautzellsystem zu testen.

Ätherisches Öl

Das ätherische Öl wurde nach Vorschrift des Europäischen Arzneibuches durch Wasserdampfdestillation (Vorlage: Xylol) aus den lyophilisierten, pulverisierten Blättern von *Rhododendron ferrugineum* L. gewonnen. Der Gesamtgehalt an ätherischem Öl betrug **7,35 mL pro kg lyophilisierter, pulverisierter Droge.**

Inhaltsstoff	% des ätherischen Öls
α-Pinen	12,7
β-Pinen	3,5
Cajepen	2,8
1,7-Di-epi-α-Cadinen	3,1
γ-Muurolen	6,5
α-Curcumen	6,1
ar-Curcumen	13,0
β-Sesbinen	12,8
α-Sesbinen	14,5
γ-Cadinen	2,5
δ-Cadinen	5,8

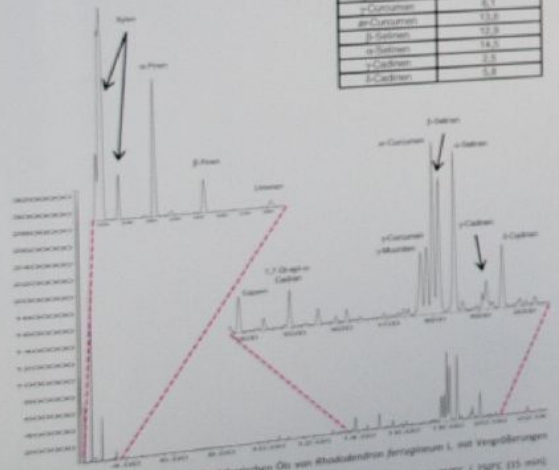


Abb. 2: Gas-Chromatogramm des ätherischen Öls von *Rhododendron ferrugineum* L. mit Vergrößerungen der relevanten Trennbereiche.
GC-MS: Temperaturprogramm: 100°C (5 min) / 3°C/min – 200°C / 10°C/min – 250°C / 250°C (15 min); Laufzeit: 58 Min.; Split: 50:1; Solvent Delay: 2 Min.; Injektor: 220°C; Säule: HP-5MS, ID 0,25 mm, Filmdicke [illegible] m, Trägergas: Helium; Detektion: MS

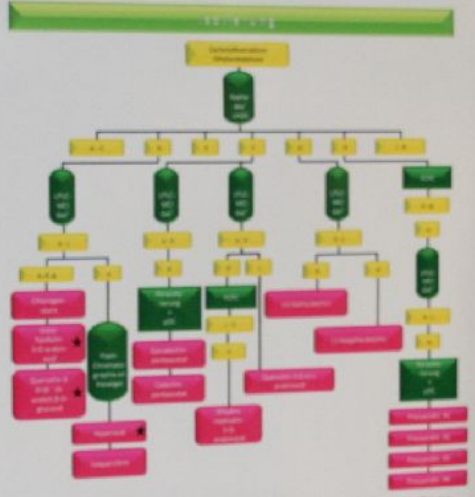
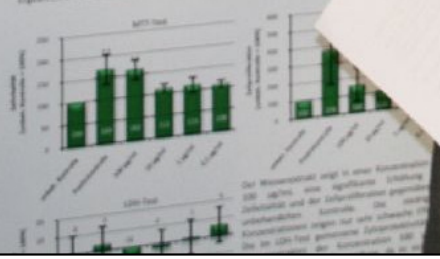
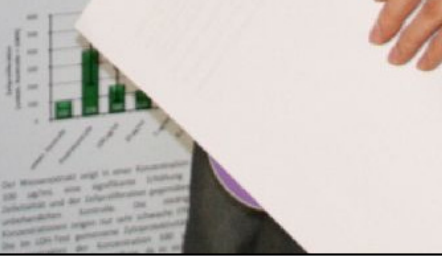
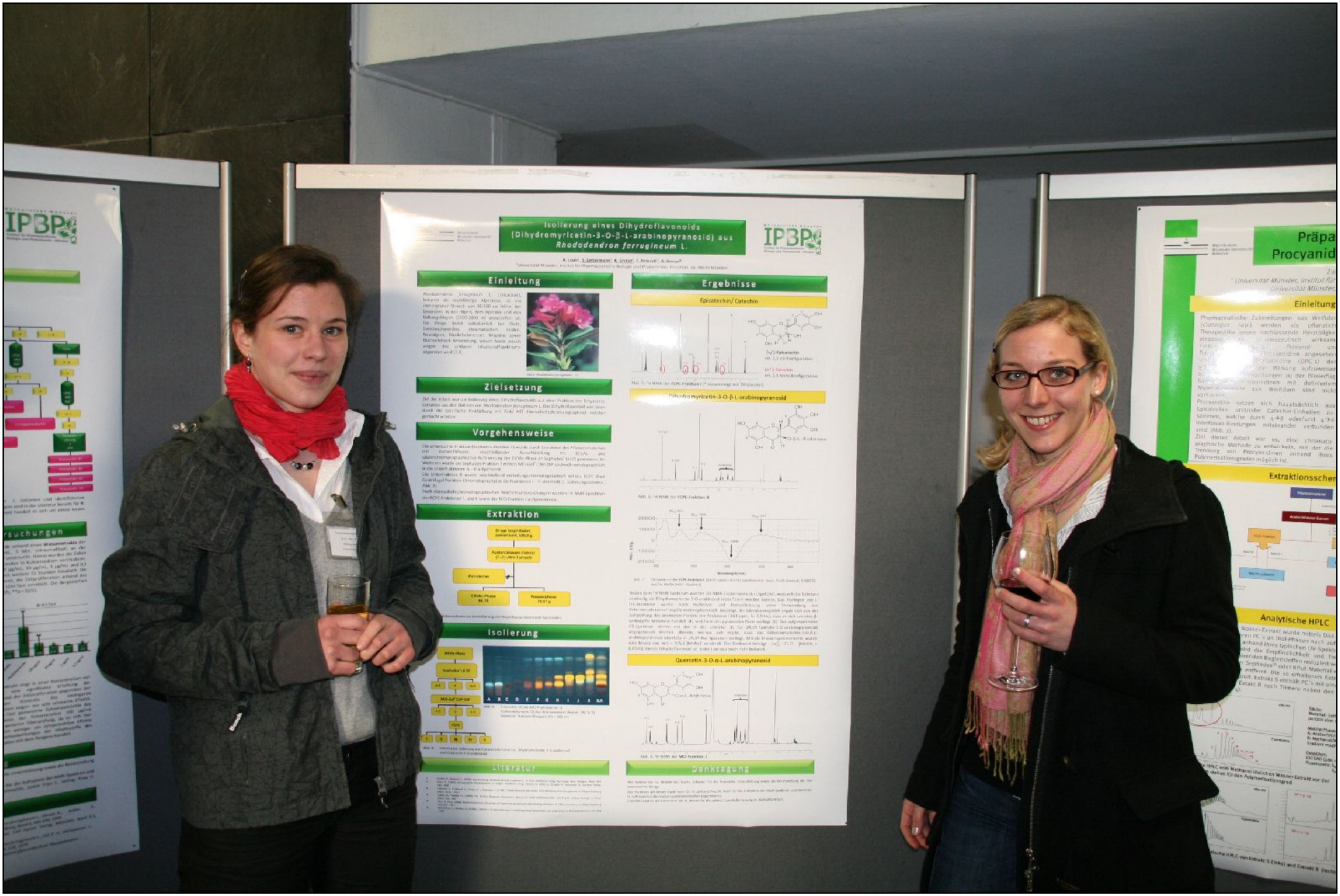


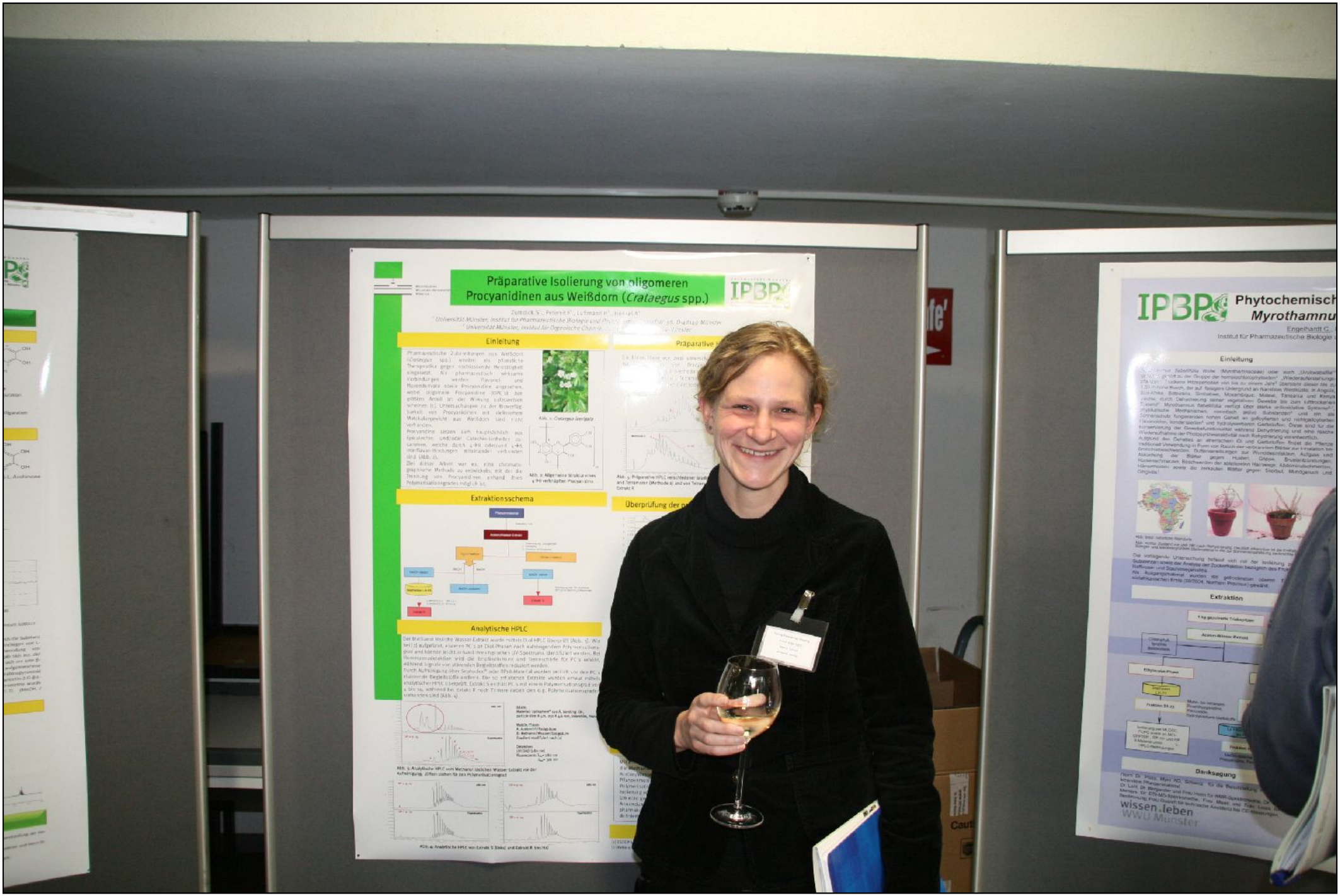
Abb. 4: Isolierungsschema der aus *Rhododendron ferrugineum* L. isolierten und identifizierten Verbindungen. Die mit einem Sternchen gekennzeichneten Verbindungen sind in der Literatur bereits für *R. ferrugineum* L. beschrieben [4]. Bei Dihydromyricetin-3-O-β-L-arabinosid handelt es sich um einen neuen Naturstoff, der in der Literatur noch nicht beschrieben wurde.

Zellphysiologische Untersuchungen

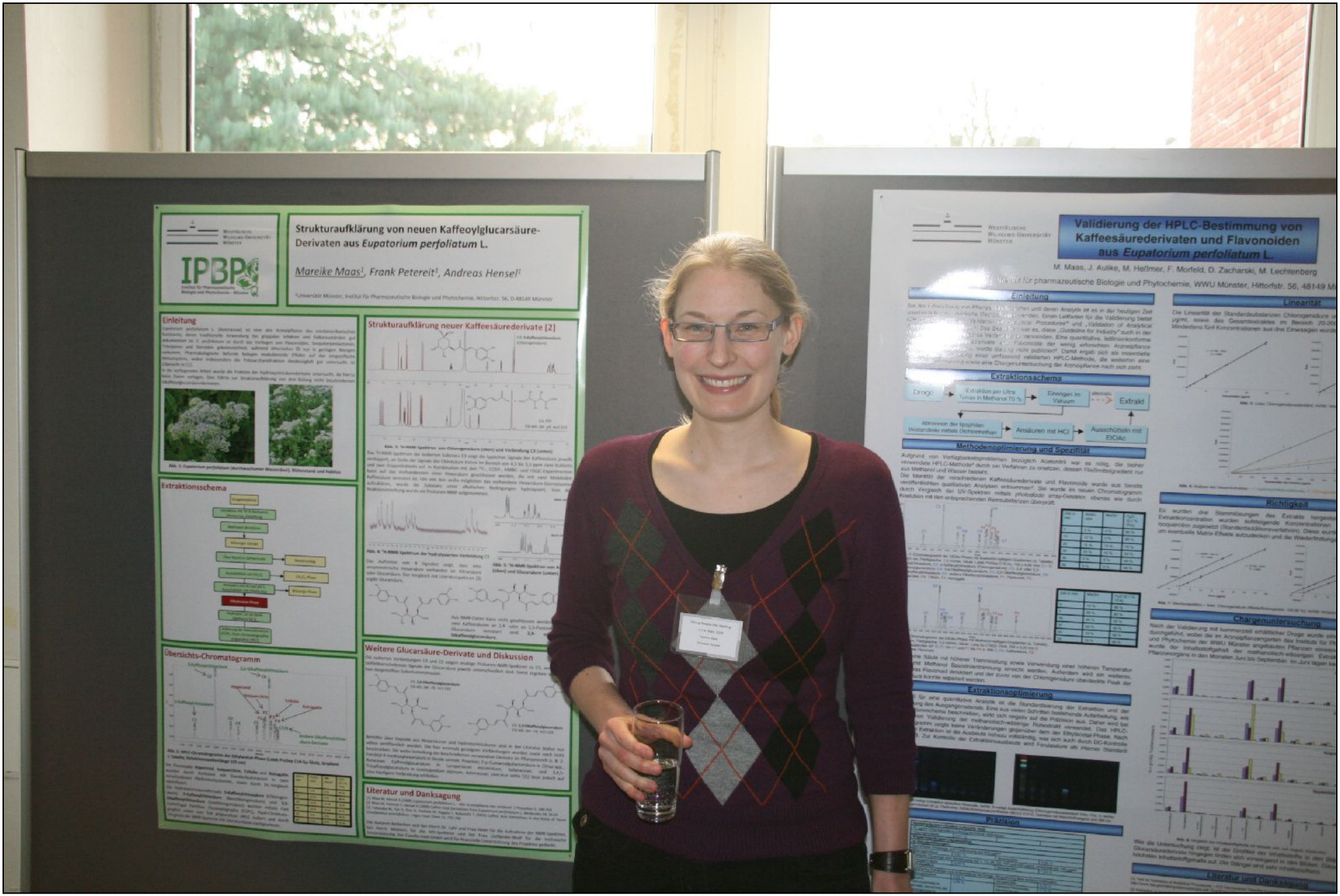





Isolierung eines Dihydroflavonoids
(Dihydromyricetin-3-O-β-L-arabinopyranosid) aus
Rhododendron ferrugineum L.
Einleitung
Zielsetzung
Vorgehensweise
Extraktion
Isolierung
Literatur
Ergebnisse
Epicatechin/ Catechin
Dihydromyricetin-3-O-β-L-arabinopyranosid
Quercetin-3-O-α-L-arabinopyranosid
Danksagung
Präpa
Procyanid
Einleitung
Extraktionsschem
Analytische HPLC


Präparative Isolierung von oligomeren Procyanidinen aus Weißdorn (*Crataegus* spp.)
IPBP
Einleitung
Extraktionsschema
Analytische HPLC
IPBP
Phytochemisch
Myrothamnu
Einleitung
Extraktion
wissen.leben
WWU Münster


von oligomeren
orn (Crataegus spp.)
IP3P
Präparative HPLC
Zusammenfassung
Literatur
Phytochemische Untersuchungen zu
Myrothamnus flabellifolia Welw.
Einleitung
Isolierte Strukturen und Ergebnisse
Diskussion


Strukturaufklärung von neuen Kaffeoylglucarsäure-Derivaten aus *Eupatorium perfoliatum* L.
Mareike Maas[1], Frank Petereit[1], Andreas Hensel[1]
[1]Universität Münster, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstr. 56, D-48149 Münster
Einleitung
Strukturaufklärung neuer Kaffeesäurederivate [2]
Extraktionsschema
Übersichts-Chromatogramm
Weitere Glucarsäure-Derivate und Diskussion
Literatur und Danksagung
Validierung der HPLC-Bestimmung von Kaffeesäurederivaten und Flavonoiden aus *Eupatorium perfoliatum* L.
M. Maas, J. Aulike, M. Heßmer, F. Morfeld, D. Zacharski, M. Lechtenberg
Einleitung
Extraktionsschema
Methodenoptimierung und Spezifität
Extraktionsoptimierung
Präzision
Linearität
Richtigkeit
Chargenuntersuchung
Literatur und Danksagung

Growth inhibiting activities of *Dipsacus sylvestris* HUDS. against *Borrelia burgdorferi* sensu stricto (*Bb*ss) in vitro

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[2] Institute for Immunology, College for Veterinary Medicine, Leipzig University, An den Tierkliniken 11, 04103 Leipzig, Germany

Abstract: Lyme borreliosis is a widespread disease of the northern hemisphere. Affected individuals are treated with a controversially discussed antibiotic-based pharmacotherapy [1]. Novel ethnobotanical approaches are based on herbal medicines, such as treatment with hydroethanolic extracts of teasel obtained from the roots of *Dipsacus sylvestris* HUDS., Dipsacaceae, although antibacterial effects of the European teasel have not been described so far [2]. In this context, solely grapefruit seed extract was tested against *Borrelia burgdorferi* in vitro without any relation to therapeutical use [3].
Fresh first year roots from *Dipsacus sylvestris* HUDS. were extracted with 70 % ethanol, ethyl acetate as well as dichloromethane. Extracts were solubilized in water (lipophilic extracts with addition of polysorbate 80) and tested for their activity against Bbss in vitro during an 8-day period. The hydroethanolic extract showed no growth inhibition whereas the two less polar fractions showed significant growth inhibiting activity. Strongest inhibition was found in the ethyl acetate extract (99.7 ± 1.0 % on day 4). The effect of polysorbate 80 on the bacterial growth was examined and found to be negligible (5.6 ± 7.6 % on day 4).

In vitro testing for growth inhibiting activities:

Figure 1: diagram of the time-dependent growth of *Bbss* (concentrations in counts/ml)

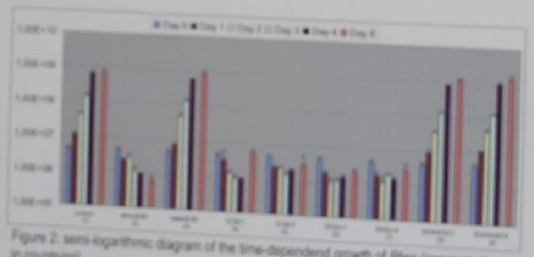

Figure 2: semi-logarithmic diagram of the time-dependend growth of *Bbss* (concentrations in counts/ml)

Legend

(1) *Bbss* growth-control
(2) antibiotic-treated culture (amoxicillin 0.5 µg/ml)
(3) Tween® 80 (polysorbate) 80-control (2.5 mg/ml)
(4/5) dichloromethane extract-treated culture (2 mg/ml, drug-extract-ratio 35:1)
(6/7) ethyl acetate extract-treated culture (2 mg/ml, drug-extract-ratio 19:1)
(8/9) hydroethanolic extract-treated cultures (2 mg/ml, drug-extract-ratio 2:1)

HPLC - fingerprint analysis and quantification of phenolic compounds in *Leonurus cardiaca* L. (Ph. Eur.) and in an antiarrhythmic refined extract

Strahler S[1], Çalış I[2], Dhein S[3], Rauwald HW[1]

[1] Department of Pharmacy, Chair of Pharmaceutical Biology, Leipzig University, Johannisallee 21-23, 04103 Leipzig, Germany
[2] Faculty of Pharmacy, Department of Pharmacognosy, Hacettepe University, 06100 Ankara, Turkey
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Abstract

A HPLC method for the fingerprint analysis and quantification of extracts from Leonuri cardiacae herba (Ph. Eur.), Lamiaceae - in particular of a refined extract with defined antiarrhythmic activity - has been developed. Using a water-methanol gradient (pH 2.1 achieved by formic acid; 1.2 ml/min; 40°C) phenylethanoid glycosides like lavandulifolioside and verbascoside, flavonoids like rutin, hyperoside, quercetin and phenolic acids such as caffeic, chlorogenic, ferulic and rosmarinic acid were separated on a RP-18 column at 330 nm. Quantification of the phenylethanoid glycosides and further phenolics was carried out using hemiarine as internal standard. In contrast to the minor groups in the extract, such as iridoids, diterpenoids and N-compounds, these major constituents are pharmacologically relevant regarding defined antiarrhythmic effects in accordance with single effects described for these compounds.

Method

For characterization of different extracts of the herb from Leonurus cardiaca (fig. 1) TLC (KG 60 F_{254} plates, Merck, Darmstadt) and HPLC (EC 250/4 NUCLEOSIL 100-5 C18 with KS 11/4 NUCLEOSIL 120-5 C18, Macherey-Nagel, Düren) analyses with the phenylethanoids lavandulifolioside and verbascoside as leading substances were performed. Beside an antiarrhythmic refined extract from our labs the H_2O- and the ethyl acetate (EA)-phases of a with CH_2Cl_2 and EA separated methanol (MeOH)-extract and the H_2O-phase of a tea after separation against $CHCl_3$ were investigated. From all examined substances chlorogenic, caffeic and ferulic acid, lavandulifolioside, verbascoside and rutin were detected in the samples and quantified by HPLC (conditions in tab. 1, 40 °C, 330 nm, relative retention times calculated by at least 40 runs in tab. 2) with hemiarine as internal standard.

Results

The investigated extracts showed for the extraction method characteristic fingerprint-chromatograms (fig. 2) and different contents of the detected phenolics (tab. 2). In the antiarrhythmic refined extract polar and/or small substances in the first ten minutes on HPLC dominated while the H_2O-phase of the MeOH-extract showed large amounts of phenylethanoids. The H_2O-phase of the tea looked like a mixture of both. Caffeic acid, ferulic acid and verbascoside were enriched in the EA-phase of the MeOH-extract. Concentrations in the herbal drug were calculated by the EA- and H_2O-phase of the MeOH-extract. In the antiarrhythmic refined extract all quantified phenolics were enriched.

Discussion

Phenolic substances in Leonuri cardiacae herba and its extracts were analysed and quantified for the first time by HPLC. The concentrations of chlorogenic

Growth

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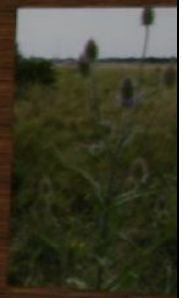


In vitro testing for gr

Figure 1: diagram of the time-d

Legend:

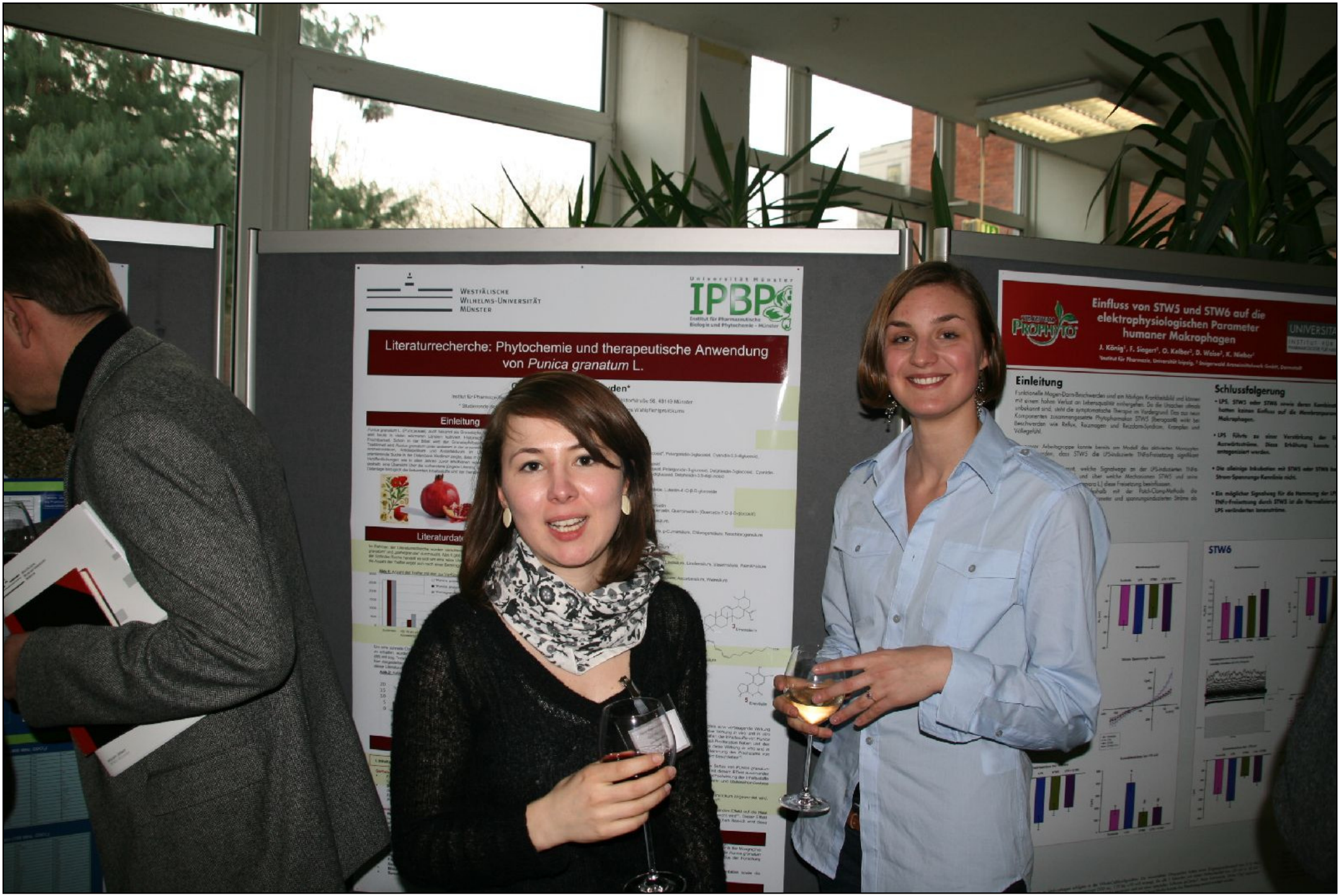
(1) Bbss growth-control
(2) antibiotic-treated culture [am
(3) Tween® 80 (polysorbate) 80
(4/5) dichloromethane extract-tr
(6/7) ethyl acetate extract-treat
(8/9) hydroethanolic extract-trea

Table 1: relative growth of Bbss c
(percentage of non-logarithmic gr

Experimen


Pharmazie


PIACENZALINE
KAIKEN
TEEWASSER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Einleitung
Einfluss von STW5 und STW6 auf die elektrophysiologischen Parameter humaner Makrophagen
Einleitung
Schlussfolgerung
STW6


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L. Heyden*
Einleit
Literatur
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3 Ursolsäure
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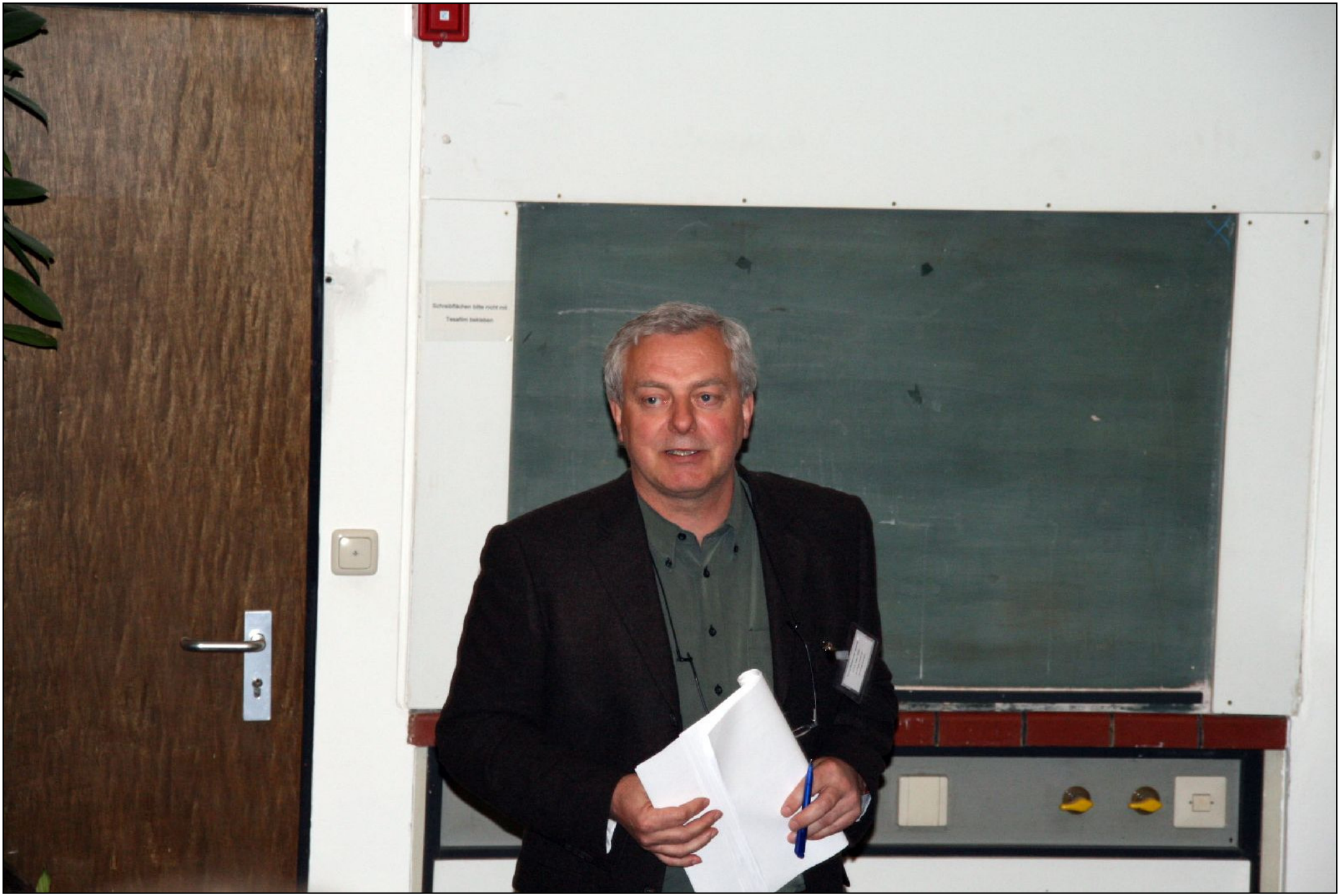

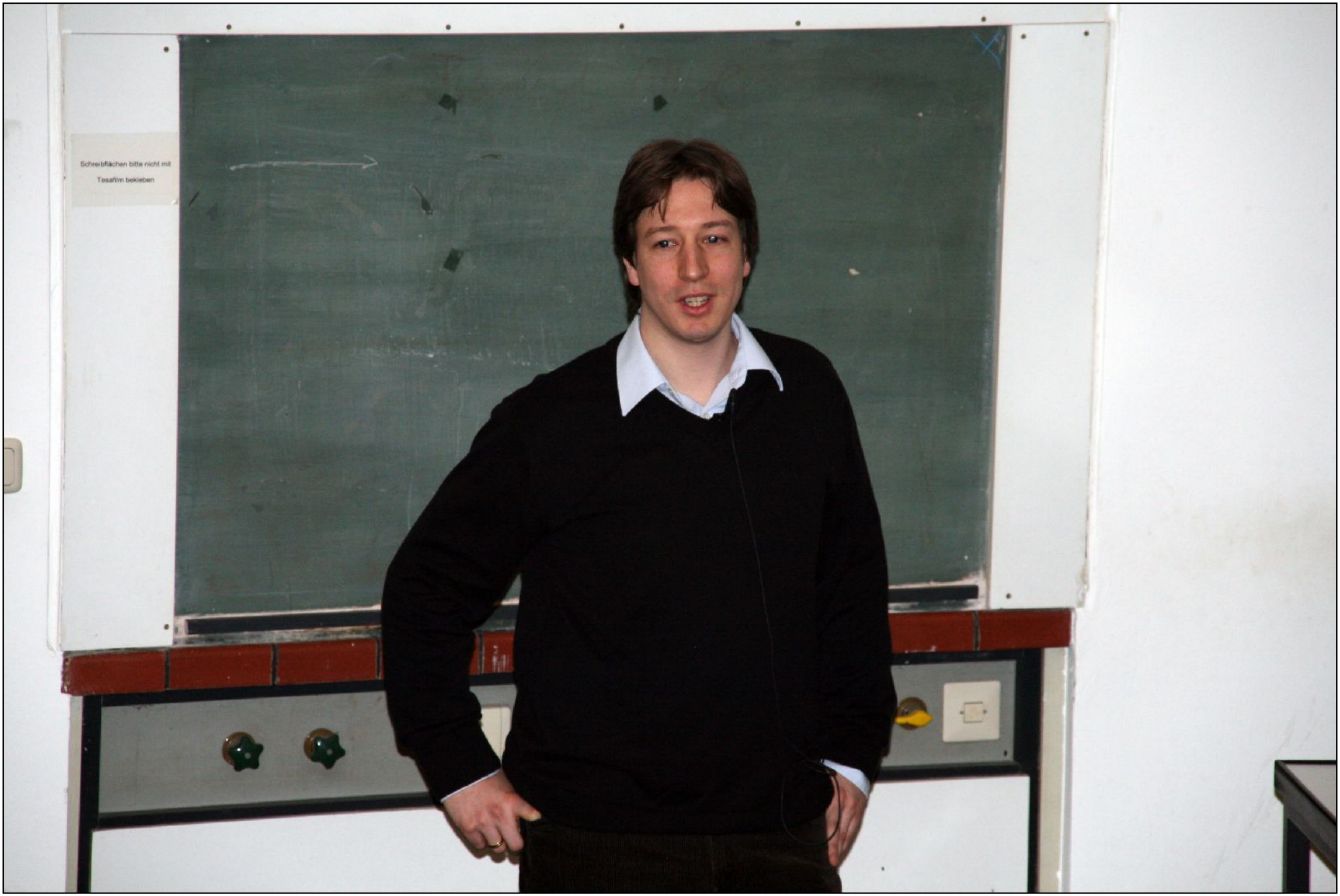
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