原著論文

Summary of the Results of the Natural Products Chemistry Research Project at the Department of Food and Nutrition of Sanyo Gakuen University-College

山陽学園大学・山陽学園短期大学における天然物化学研究プロジェクトの業績一覧

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ケニー・クフタ [1]

私は、ドイツのライプチヒ大学で、日本の漢方医学を基にした植物化学及び薬用植物の薬理学について、博士課程の研究プロジェクトを完了した後、(この研究で私はベルリンの日本大使館によってドイツ語日本語友好賞 2011 を受賞した)。当時の山陽学園大学学長で医学博士である赤木忠厚先生によって、補完代替医学の教育・研究を目的として 2012 年春に新たに設立された山陽学園大学補完・代替医療教育研究センターの専任講師として招待された。

2013 年春に新学長實成文彦先生によって、山陽学園大学の教育及び研究政策が再編成され、私の研究部署は山陽学園短期大学、食物栄養学科、天然物化学研究に変更になった。

ライプチヒ大学と山陽学園のどちらにおいても、私は継続的に、互いに学びあう西洋と日本の両方で医師を支援し、東と西の両方で患者の健康と福祉を強化するため、日本の伝統的な漢方医学とヨーロッパの伝統的な薬用植物学の間のギャップを埋めようとに努力してきた。

過去 3 年の間に、私は日本の中でも世界でも、山陽学園と多数の大学間の国内及び国際的な学術協力を確立することができた。私の母校ライプチヒ大学(ドイツ)に加えて、岡山理科大学(岡山)、明治国際医療大学(京都)、長崎国際大学(長崎)、及び長崎大学(長崎)日本における島根大学(出雲)だけでなく、ヌエボ・レオン州立自治大学(メキシコ)、広州中山大学(中国)、広州済南大学(中国)、浙江省農業・林業大学(中国)、ネゲブのベングリオン大学(イスラエル)、コペンハーゲン大学(デンマーク)、ミュンスター大学(ドイツ)など世界中の大学で活動を行うことができた。

これらのプロジェクトの大半はまだ進行中であるが、過去 3 年間、すでに私の研究は国際薬用植物研究雑誌における様々な雑誌に論文を受理され、数々の国際科学会議で研究データの多数を日本語、英語、ドイツ語で口頭発表した。

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昨年 2014 年に、サンクトペテルブルク（ロシア）の薬用植物に関する国際会議とギマランイス（ポルトガル）における国際薬用植物天然産物研究学会（GA）の年次会議における研究結果のプレゼンテーションは、この年の私の研究で、最も重要なものであった。

これらの国際会議で、私は山陽学園大学での天然物化学研究プロジェクトの成果を説明し、サンクトペテルブルクでのロシア科学アカデミー薬学部門ポスター発表賞と、薬用植物及び天然産物の研究学会のエゴン・スタール賞メダルをそれぞれ受賞した。

以下のページでは、私は私の山陽学園で最も成功したプロジェクトと、それに対応する研究成果の簡単な説明が含まれている。これらの研究活動のほかに、私は、ヨーロッパでは日本の伝統的な漢方医学の使用と、欧州における伝統薬用植物学の使用を広めるためにも、時間を費やした[1]。

特にこの後者の点では、私は、西洋医学で治療する際のハーブの使用方法について、長崎国際大学では客員教授、金沢大学と京都府立医科大学では客員講師として、一連の講義を受け持っている。

After completing my doctoral research project on the phytochemistry and pharmacology of medicinal plants from Japanese Kampo medicine at Leipzig University in Germany – for which I was awarded the German Japanese Friendship Award 2011 by the Japanese embassy in Berlin – I was approached by the chancellor of Sanyo Gakuen University – Prof. Dr. T. Akagi – to join the newly established "Teaching and Research Center for Complementary and Alternative Medicine"（補完代替医療教育研究センター）starting in Spring 2012. After a reorientation of the teaching and research policy at Sanyo Gakuen by the new chancellor Prof. Dr. F. Jitsunari in spring 2013, my research positon was reassigned to the department of food and nutrition of Sanyo Gakuen University-College as the project for "Natural Products Chemistry Research"（山陽学園短期大学、食物栄養学科、天然物化学研究）.

Both during my time at Leipzig University and at Sanyo Gakuen I have continuously strived to bridge the gap between traditional Japanese Kampo medicine and European Traditional Medicinal Herbalism in order to help doctors both in the West and in Japan to learn from each other and therefore to enhance the health and wellbeing of the patients in both East and West.

During the past three years, I was able to establish national and international academic cooperation between Sanyo Gakuen and numerous universities both inside Japan and around the world. Besides my alma mater Leipzig University (Germany), these are Shimane University (Izumo), Okayama University of Science (Okayama), Meiji University of Integrative Medicine (Kyoto), Nagasaki International University (Nagasaki), and Nagasaki University (Nagasaki) in Japan, as well as Universidad Autónoma de Nuevo León (Mexico), Sun Yat-sen University Guangzhou (China),

[1] フォルカー・フィンテルマン、ルードルフ・フリッツ・ヴァイス、三浦於菟、林真一郎、ケニー・クフタ、田中耕一郎: 植物療法フィトセラピー事典:産調出版株式会社,2012.

Jinan University Guangzhou (China), Zhejiang Agricultural & Forestry University (China), Ben-Gurion University of the Negev (Israel), University of Copenhagen (Denmark), and University of Münster (Germany) around the world.

Whereas the majority of these projects are still ongoing, the past three years have already resulted in a multitude of research data which I published in a series of articles in international medicinal plant research journals and presented in oral communications in Japanese, English, and German language at numerous international scientific conferences.

During the past year 2014 the presentations of these results at the international conference PHYTOPHARM in St. Petersburg (Russia) and at the annual conference of the Society for Medicinal Plant and Natural Product Research in Guimarães (Portugal) formed the highlights of my academic year. At these conferences, the below described results of my "Natural Products Chemistry Research" project at Sanyo Gakuen University-College were awarded the poster presentation price of the Russian academy of Pharmacy at St-Petersburg and the Egon Stahl Award Medal of the Society for Medicinal Plant and Natural Product Research, respectively.

On the following pages, I have included short descriptions of my most successful projects here at Sanyo Gakuen and the corresponding research results.

Besides these research activities, I have also tried to use some of my time to spread the use of traditional Japanese Kampo medicine in Europe and the use of European Traditional Medicinal Herbalism in Japan [1]. Especially in this latter respect, I was made Visiting Professor at Nagasaki International University and Visiting Lecturer at both Kanazawa University and Kyoto Prefectural University of Medicine, where I have given a series of lectures on the therapeutic use herbs of Western medicine.

Egon Stahl Award ceremony, 1st of September 2014, Guimarães, Portugal

Egon Stahl Medal

Open-label clinical pilot-study on an herbal multi-component topical TCM therapy for atopic dermatitis and comorbid conditions

We recently reported the efficacy of a novel TCM therapy – consisting of both oral and topical medications – against atopic dermatitis (AD) [1,2,3].

Building on these results, we developed improved galenic formulations of two multi-component TCM extracts (overall 13 drugs, see Table 1) for purely topical application.

All drugs were powdered and extracted in boiling water for 5 h. Extract 1 was formulated as an herbal soap (1) with anti-inflammatory activity and used for washing the affected areas. For extract 2, three distinct galenic formulations were developed, namely a lotion (2A) with high skin penetrating activity and fast-acting antipruritic effect; a gelatinous jelly (2B) favorable for the treatment of scratching and rupture scars; and a Vaseline based ointment (2C).

In this open-label clinical pilot-study 129 AD patients were continuously treated with these topical TCM preparations. For evaluating their clinical efficacy, standardized scores were used for the severities of both AD (clinical severity 0-4) and pruritus (pruritus score 0-4).

Both scores had significantly improved at the end of treatment after two months. Additionally, empirical clinical data on the therapeutic efficacy concerning related comorbid conditions like psoriasis, acne, alopecia, as well as fungal, bacterial, and viral skin infections were collected, demonstrating high therapeutic potentials in all these conditions. None of the tested preparations did display any significant adverse effects, facilitating even prolonged application on newborn infants.

The presented topical TCM preparations can be used safely and effectively in both clinical AD therapy, self-medication of skin disorders, or even as cosmetics.

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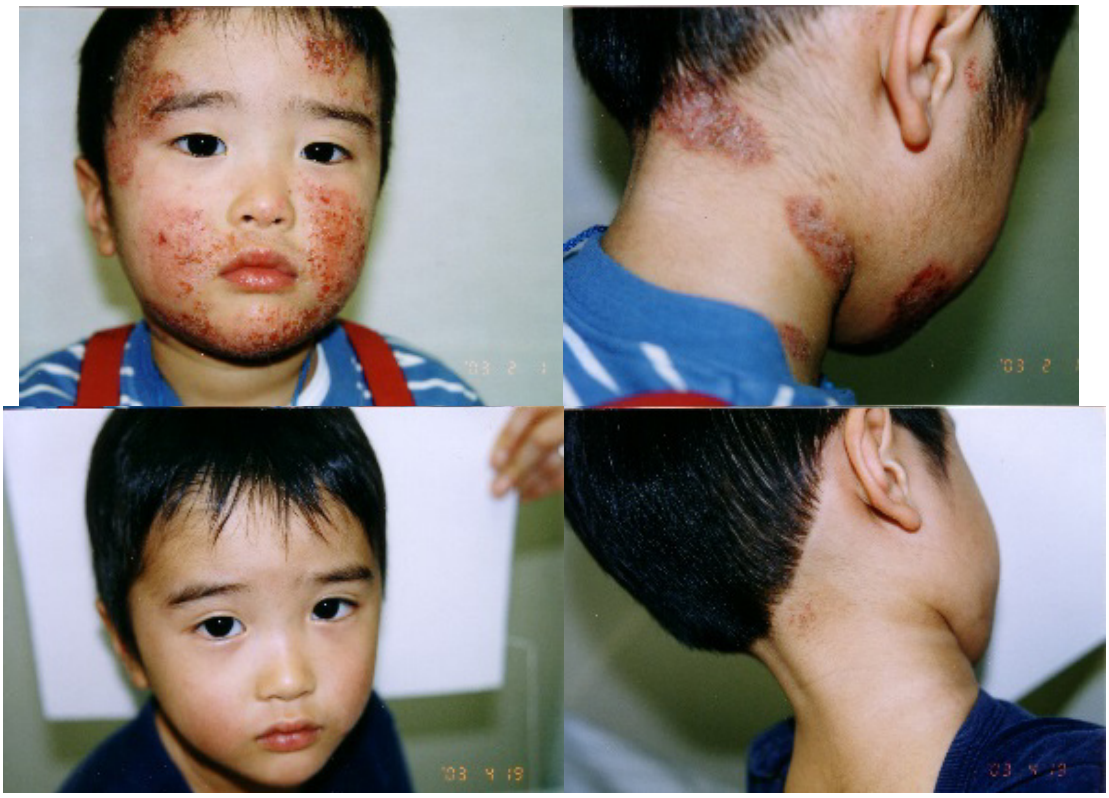
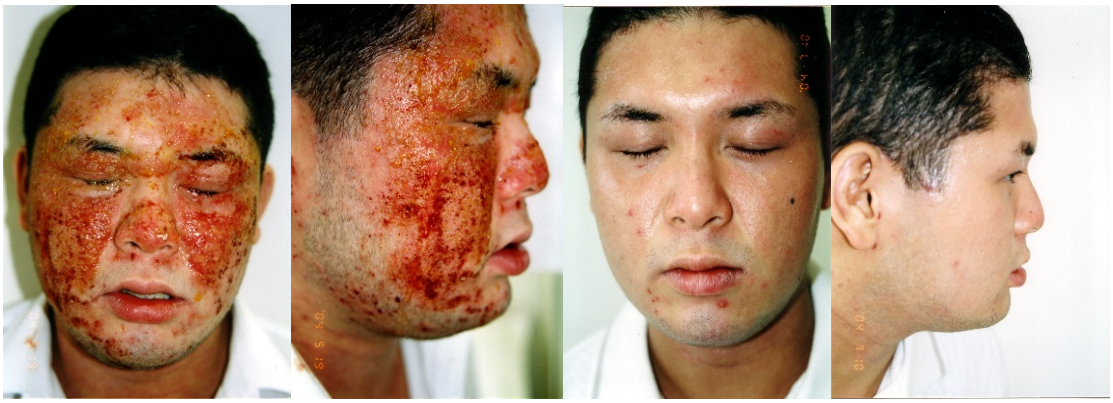
[2] Li S, Kuchta K, Tamaru N, Lin Y, Iwasaki S, Wang R, Kobayashi Y, Rauwald HW, Kamei T: Efficacy of a Novel Herbal Multicomponent Traditional Chinese Medicine Therapy Approach in Patients with Atopic Dermatitis. Forsch Komplementmed 2013; 20: 189-196

[3] 李頌華、田丸直美、林宇、王如偉、ケニー・クフタ、佐藤直人、岩崎純夫、小林裕太：興味深い臨床報告　アトピー性皮膚炎 102 例における複方苦参製剤の外用効果。中医臨床　35: 46-50, 2014.



Before onset of therapy, both face and neck were covered with striking erythema and eczema, accompanied by ulceration of large skin areas. After 2 months of therapy, the patient's condition has remarkably improved.

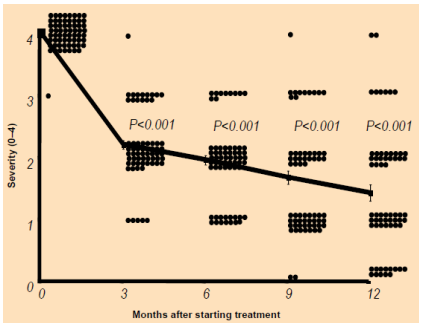


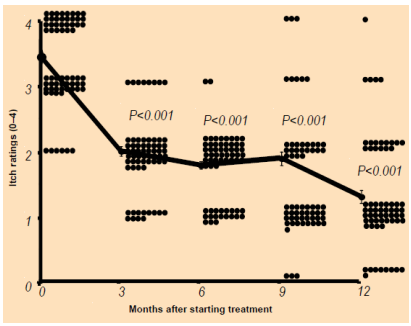
Before treatment, the face displayed severe erythema and ulceration accompanied by abundant bleeding.

After 2 months of treatment, remarkable improvement is obvious.

TCM plant drugs used in extract 1, extract 2, or both of them, given together with their traditional indications for external use according to Chin.Ph.; Luo HS, Luo DH. Immune Chinese Medicine. Beijing Medical University and China Union Medical University Press, Beijing 1999; and Huang KT, Ding ZZ, Zhao SX. Modern Compendium of Materia Medica. Chinese Medical Science and Technology Press, Beijing 2001.

Extract	Scientific Name	Plant Part	Japanese - Chinese - Name	Pharmacological Effect (TCM)	TCM Indications (External)	Drug material per litre
2	*Angelica sinensis*	root	トウキ 当帰	improves blood circulation	eczema, frostbite	10 g
1	*Chrysanthemum indicum*	flowers	キクカ 菊花	anti-inflammatory	eczema, skin infection, cervicitis, mumps	12 g
2	*Citrus x limon*	fruit juice	レモン 檸檬	skin protective, cosmetic, antiseptic, antibiotic, deodorizing	skin protective, frostbite, cosmetic, insect repellent	5 ml
1	*Coptis chinensis*	rhizome	オウレン 黄連	anti-inflammatory, heals ulcer, antibacterial	eczema, skin infection, otitis externa, aphthous ulcer	12 g
2	*Dryobalanops aromatica*	resin	ヒョウヘン 氷片	anti-inflammatory, antibacterial, analgesic, relieves itching, promotes skin permeation of other extracts	pruritus, skin ulcers, eczema, periodontitis, otitis, hemorrhoids	10 g
2	*Glycyrrhiza uralensis*	root	カンゾウ 甘草	anti-inflammatory, heals ulcer, antiallergenic	eczema, psoriasis, skin ulcer	10 g
2	*Isatis tinctoria*	leaves	ダイセイヨウ 大青葉	antipyretic, antibacterial, antiviral	chronic eczema, epidemic mumps	20 g
1	*Paris polyphylla*	rhizome	シチヨウイッシカ 七叶一枝花	anti-inflammatory, detumescent, strongly antibacterial, anti-infective	eczema, bruising, swellings, mumps, mastitis, snake and insect bites	12 g
2	*Polygonum cuspidatum*	rhizome	コジョウ 虎杖	heals ulcer, haemostatic, antibacterial, anti-infective	suppurative dermatitis, bleeding wounds, scalding, snake bites	20 g
1	*Punica granatum*	pericarp	セキリュウヒ 石榴皮	astringent, heals ulcer, skin protective, antimicrobial, antifungal, antiviral	suppurative otitis media, skin ulcer	12 g
1+2	*Scutellaria baicalensis*	root	オウゴン 黄芩	anti-inflammatory, antiallergenic, antibacterial, antiviral	eczema, psoriasis, hemorrhoids	12 g / 20 g
1+2	*Sophora flavescens*	root	クジン 苦参	antiallergenic, antipyretic, antifungal	eczema, skin infection, pruritus vulvae	12 g / 20 g
1	*Stemona sessilifolia*	root	ビャクブコン 百部根	relieves itching, antibacterial, antiviral	skin infection, trichomoniasis, lice	12 g





Age range, years (mean)	0–70 (18.2)[a]
	n
All patients	94[b]
Duration of symptoms, years	
0–9	33
10–20	39
20≥	22
Severity	
Mild (1)	18
Moderate (2)	35
Severe (3)	21
Most severe (4)	20
Steroid use at initial diagnosis	38

[a]Male = 18.6; female = 17.8.
[b]Male = 49; female = 45.

Effect	n	%
Markedly improved (sum of the 2 scores decreased by 4 or more)	32	34
Improved (sum of the 2 scores decreased by 2 to 3)	59	63
Slightly improved (sum of the 2 scores decreased by 1)	3	3
Ineffective (sum of the 2 scores decreased by 0)	0	0

Clinical score was quantitatively defined according to the guidelines of the Japanese Dermatological Association.

A phytosterol enriched refined extract of *Brassica campestris* L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model [4]

In Qinghai Province, the *Brassica campestris* L. pollen preparation Qianlie Kang Pule'an Tablets (QKPT) is traditionally used for benign prostatic hyperplasia (BPH) therapy. However, in QKPT the content of supposedly active phytosterols is relatively low at 2.59%, necessitating high doses for successful therapy.
A phytosterol enriched (4.54%) refined extract of *B. campestris* pollen (PE) was developed and compared with QKPT in a BPH rat model.

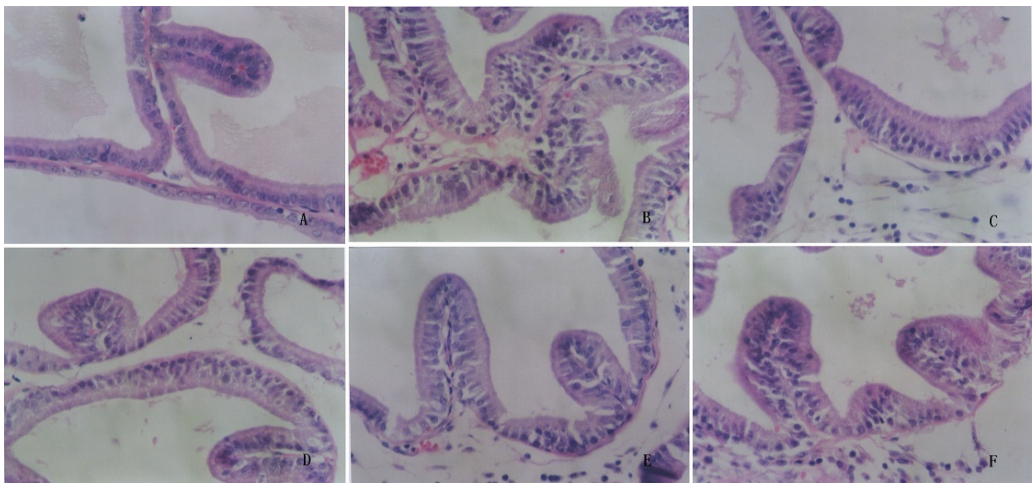
Six groups of rats (n=8 each), namely sham operated distilled water control, castrated distilled water control, castrated QKPT 2.0g/kg, castrated PE 0.1g/kg, castrated PE 0.2g/kg, and castrated PE 0.4g/kg were intragastrically treated with the respective daily dose. Testosterone propionate (0.3mg/day) was administered to all castrated rats, while the sham operated group received placebo injections. After 30 days, the animals were sacrificed and prostates as well as seminal vesicles excised and weighted in order to calculate prostate index (PI) and seminal vesicle index (SVI), defined as organ weight in g per 100 g body weight.

Compared with sham-operated controls, both PI ($p<0.01$) and SVI ($p<0.01$) were significantly increased in all castrated rats. After treatment with PE at 0.4 and 0.2 g/kg or QKPT at 2.0 g/kg per day, both indices were significantly reduced ($p<0.01$) as compared to the castrated distilled water control. For PE at 0.1 g/kg per day only PI was significantly reduced ($p<0.05$). At the highest PE concentration of 0.4 g/kg per day both PI and SVI were also significantly reduced when compared to the QKPT group ($p<0.05$).

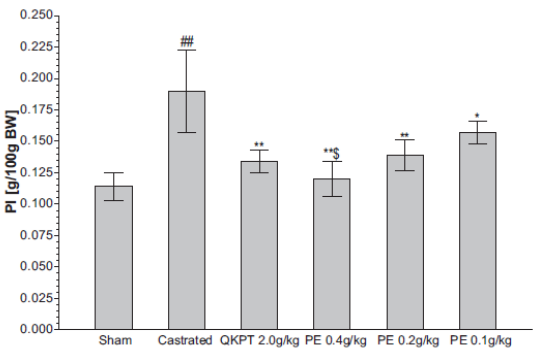
Both PE and QKPT demonstrated curative effects against BPH in the applied animal model. In its highest dose at 0.4 g/kg per day, PE was clearly superior to QKPT.

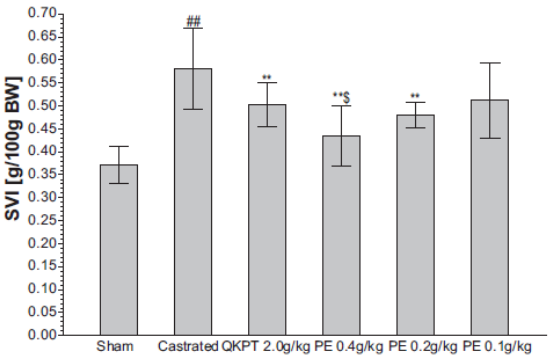
[4] Ruwei Wang, Yuta Kobayashi, Yu Lin, Hans Wilhelm Rauwald, Ling Fang, Hongxiang Qiao, Kenny Kuchta: A phytosterol enriched refined extract of Brassica campestris L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model as compared to the classical TCM pollen preparation Qianlie Kang Pule'an Tablets. Phytomedicine 22: 145-152, 2015.

Flowering *Brassica campestris* fields in Chengdong, Qinghai Province (青海省), China (left); this region is also famous for bee farming. The pollen is collected from the flowers by the bees (right) and later harvested from their hives.



Histomorphologic changes in the rat prostates stained with haematoxylin and eosin (magnification: x400). Sham operated distiled water control group (A), castrated distilled water control group (B), castrated QKPT 2.0g/kg group (C), castrated high-dose PE 0.4g/kg group (D), castrated mid-dose PE 0.2g/kg group (E), and castrated low-dose PE 0.1g/kg group (F).





Left: Bar chart of the measured values for the prostate index (PI) among the individual groups of experimental animals (n = 8) in this study, displaying high reproducibility of the physiological effects of the examined pollen preparations. ##p < 0.01 as compared with sham-operated distilled water control group; *p < 0.05; **p < 0.01 as compared with castrated distilled water control group; $p < .05 as com-pared with castrated QKPT 2.0 g/kg group.

Right: Bar chart of the measured values for the seminal vesicle index (SVI) among the individual groups of experimental animals (n = 8) in this study, displaying high reproducibility of the physiological effects of the examined pollen preparations. ##p < 0.01 as compared with sham-operated distilled water control group; **p < 0.01as compared with castrated distilled water control group; $p < 0.05 as compared with castrated QKPT 2.0 g/kg group.

Curcumin induces apoptosis in hepatic stellate cells by modulating the expression of apoptosis related growth factors

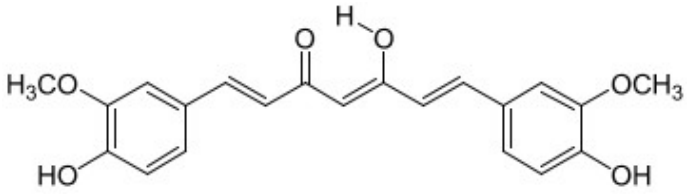
Turmeric (*Curcuma longa* L.) and its phenolic constituent curcumin exert both preventive and curative effects on hepatic fibrosis in rat-models, inducing apoptosis and inhibiting proliferation of hepatic stellate cells (HSC).

Cultured rat HSCs (HSC-T6) were incubated with 10, 20, 30, and 40 μM of curcumin for 24 h, after which apoptosis was measured by flow-cytometry in comparison to a negative-control cultured without curcumin treatment. Expression of the pro-apoptotic factors Fas and p53b as well as the anti-apoptotic factor Bcl-2 was monitored by immunocytochemical ABC staining.

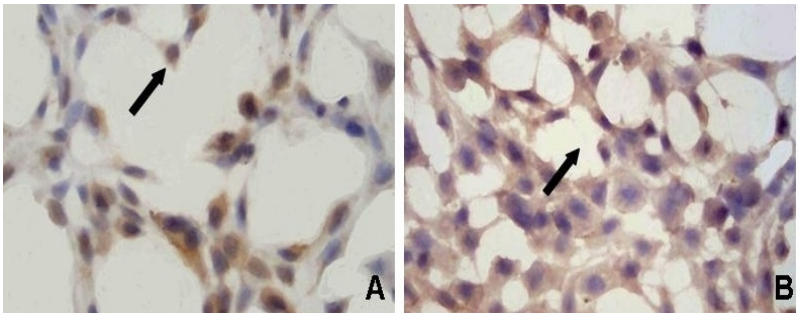
After incubation with 20, 30, or 40 μM curcumin for 24 h, the respective apoptosis indices were 11.6±2.8%, 52.0±4.4%, and 87.1±22.6%, all significantly higher than the negative-control group with 3.8±0.6% ($p<0.01$). Incubation with 10 μM yielded 6.5±1.88% thus not exhibiting the same level of statistical significance. After incubation with 20 μM curcumin for 24 h, the expression rates of the pro-apoptotic factors Fas and p53 were increased to 87.4±2.8% and 43.1±7.3%, as compared to the respective negative-control values of 53.1±5.1% and 20.6±7.2%. The expression rate

of the anti-apoptotic factor Bcl-2 was decreased to 28.7±5.9% as compared to the negative-control with 95.4±3.6%. All these effects of 20 μM curcumin were highly reproducible ($p<0.05$), in contrast to those of the 10 μM sample. In the case of the 30 and 40 μM samples, apoptosis occurred so rapidly that the ABC staining could not be carried out properly.

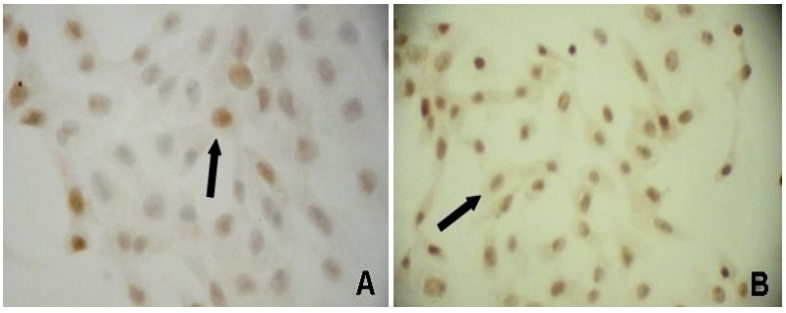
Curcumin has an up-regulating effect on pro-apoptotic factors like Fas and p53 as well as a down-regulating one of the anti-apoptotic factor Bcl-2, thus inducing apoptosis in HSC.



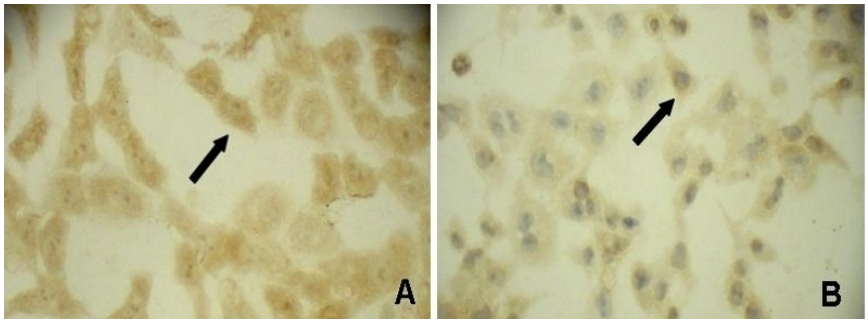
クルクミン / Curcumin



Expression of Fas (200 X). (A) HSCs of the control group received no treatment. (B) HSCs treated with 20 μM curcumin. Brown gains, which reveal expression of Fas in both membrane and cytosol (arrows), are significantly more numerous in B, proving an increase in expression of Fas as compared to the control group.



Expression of p53 (200 X). (A) HSCs of the control group received no treatment. (B) HSCs treated with 20 μM curcumn. Brown grains, which reveal expression of p53 in the nuclei (arrows), are significantly more numerous in B, proving an increase in expression of p53 as compared to the control group.



Expression of Bcl-2 (200 X). (A) HSCs of the control group received no treatment. (B) HSCs treated with 20 μM curcuin. Brown grains, which reveal expression of Bcl-2 at the cell membrane (arrows), are significantly more numerous in A, proving an decrease in expression of Bcl-2 as compared to the control group.

Expression rates of Fas, p53, and Bcl-2 following curcumin treatment (20 μM).
* = P < 0.05 as compared with the control group

Groups	Expression rate		
	Fas	p53	Bcl-2
Control group	33.1 ± 3.1 %	20.6 ± 7.2 %	95.4 ± 3.6 %
Curcumin group	87.4 ± 2.8 %*	43.1 ± 7.3 %*	28.7 ± 5.9 %*

Randomized cross-over trial on mood enhancing effects of bergamot (*Citrus bergamia* (Risso) Wright & Arn.) volatile oil vapor, also regarding personality and lifestyle related changes in salivary cortisol levels [5,6]

Bergamot essential oil (BEO) is widely used in aromatherapy for chronic stress and anxiety.

As clinical data for these indications are still insufficient, we conducted a randomized cross-over trial in human subjects.

Data were collected under three testing conditions – rest (R), rest + water vapor (RW), rest + water vapor + BEO (RWB) – for 15 min each in 42 healthy women. Heart rate variability was recorded continuously, calculating its high-frequency component (HF), and saliva samples were collected for determination of salivary

[5] 渡邉映理、木村真理、ケニー・クフタ、亀井勉、今西二郎：ベルガモット精油による芳香浴の自律神経系および情動に及ぼす効果。Aroma Research 54: 150-154, 2013.

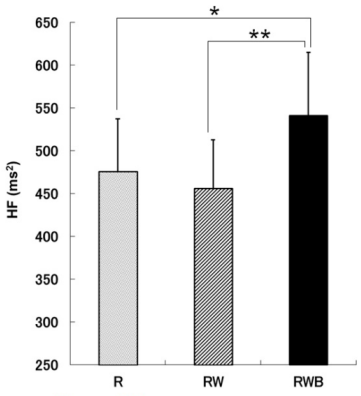
[6] Eri Watanabe, Kenny Kuchta, Mari Kimura, Hans Wilhelm Rauwald, Tsutomu Kamei, Jiro Imanishi: Effects of bergamot (Citrus bergamia (Risso) Wright & Arn.) essential oil aromatherapy on mood states, parasympathetic nervous system activity, and salivary cortisol levels in 41 healthy females. Research in Complementary Medicine 22: 43-49, 2015.

cortisol (CS) levels via ELISA after each test, while the subjects were given 10 min of rest and an additional 10 min to fill out psychological questionnaires.

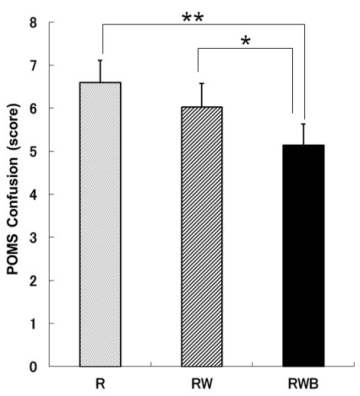
In comparison to the pure water placebo, the BEO vapor data show a significant increase in HF heart rate components (p=0.009), indicating increased activity of the autonomous nervous system, as well as a decrease in confusion (p=0.035) and fatigue (p=0.042), paired with enhanced vigor of mood (p=0.017). CS levels decreased in 32 subjects (CS-D group) but increased in the remaining 10 (CS-I group). When comparing the lifestyles of this CS-I group with those of the of the CS-D group, the former were significantly more ordered (t=-2.08, p=0.044) and values for extraversion (t=1.92, p=0.048), and optimism (t=2.17, p=0.035) were higher, while their feelings of fatigue (t=-2.61, p=0.012) were lower.

BEO, when inhaled after dispersion in water vapor, exhibits swift psychological and physiological effects favorable for the therapy of chronic fatigue, stress, and anxiety. These effects are influenced by the individual lifestyle of the subject.

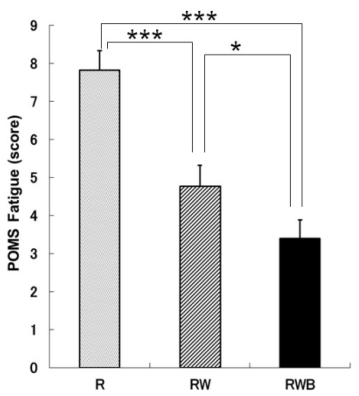
Bergamot (Citrus bergamia (Risso) Wright & Arn.): Photography (left) and illustration from a 19th century German herbal (Köhler's Medizinal-Pflanzen Atlas, 1887) (middle). In the present randomized cross-over trial, we used Bergamot essential oil (BEO), which was dispersed in the air together with water vapour using an ultrasound diffusor from “Seikatsu no Ki” (right).



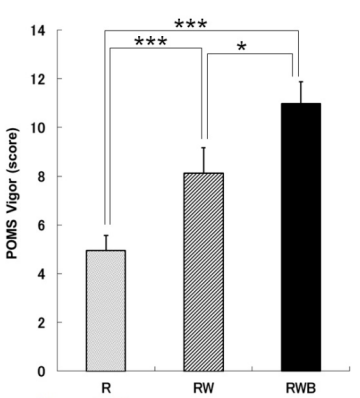
n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
1. HF heart rate components



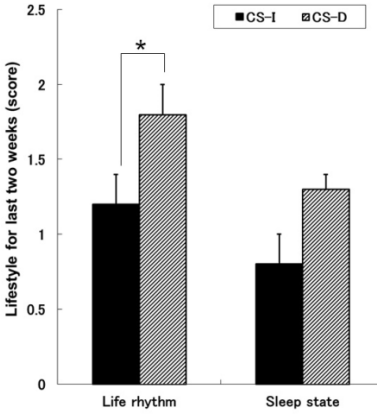
n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
2. Confusion values in the profile of mood state questionnaire (POMS)



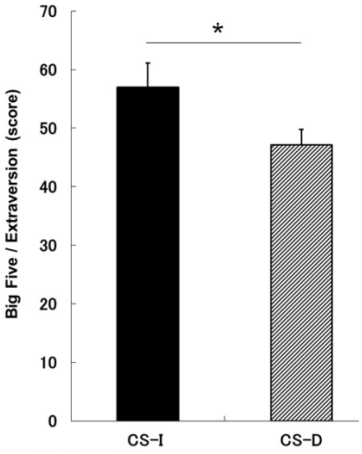
n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
3. Fatigue values in the profile of mood state questionnaire (POMS)



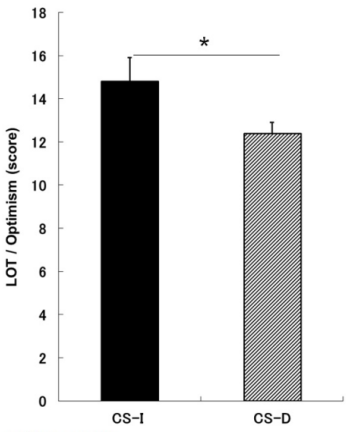
n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
4. Vigor of mood values in the profile of mood state questionnaire (POMS)



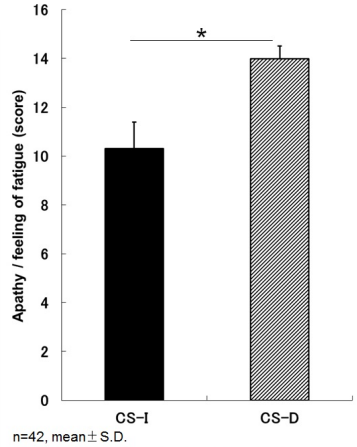
n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
5. Values for ordered lifestyle



n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
6. Values for extraversion



n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
7. Values for optimism



n=42, mean±S.D.
Paired t test, p<0.001***, p<0.010**, p<0.050*
8. Values for feelings of fatigue

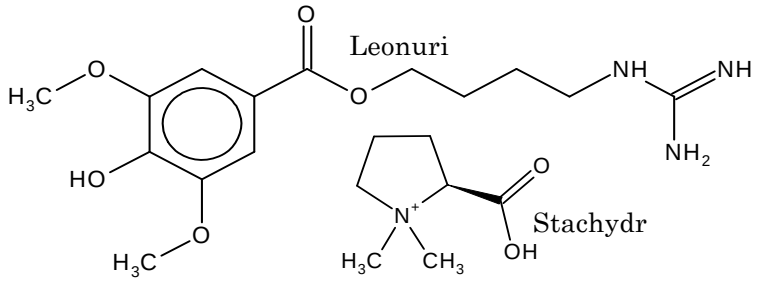
Effects of *Leonurus japonicus* Houtt. and its N-containing constituents leonurine and stachydrine on the activity of PPARα, ß/δ, and γ in a newly developed *in vitro* luciferase reporter gene assay

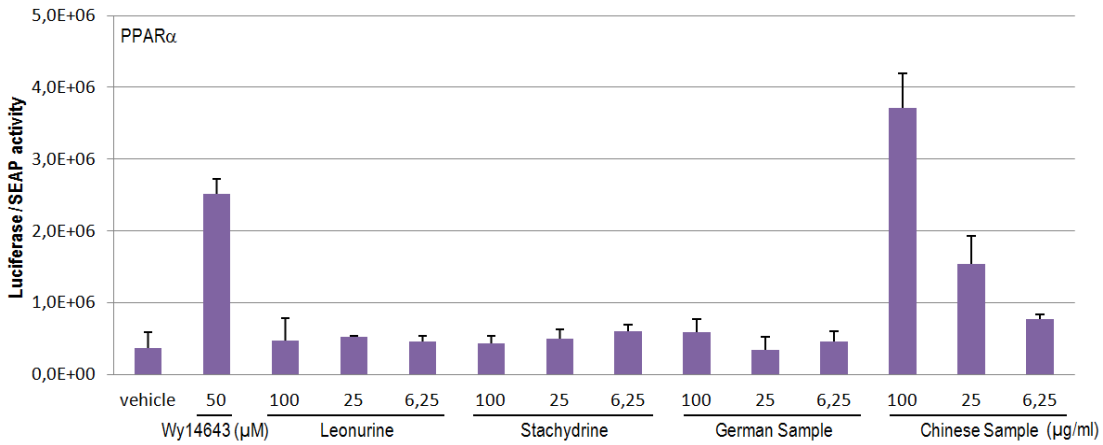
Leonurus japonicus (Yimucao; Chin.Ph., DAB) has been used in TCM since earliest times for conditions presently referred to as the "metabolic syndrome". Here, the agonistic activity of aqueous Yimucao extracts – both from China and from German TCM plant cultivation – and their dominant constituents leonurine and stachydrine [7,8] on the metabolic syndrome related peroxisome proliferator-activated receptors

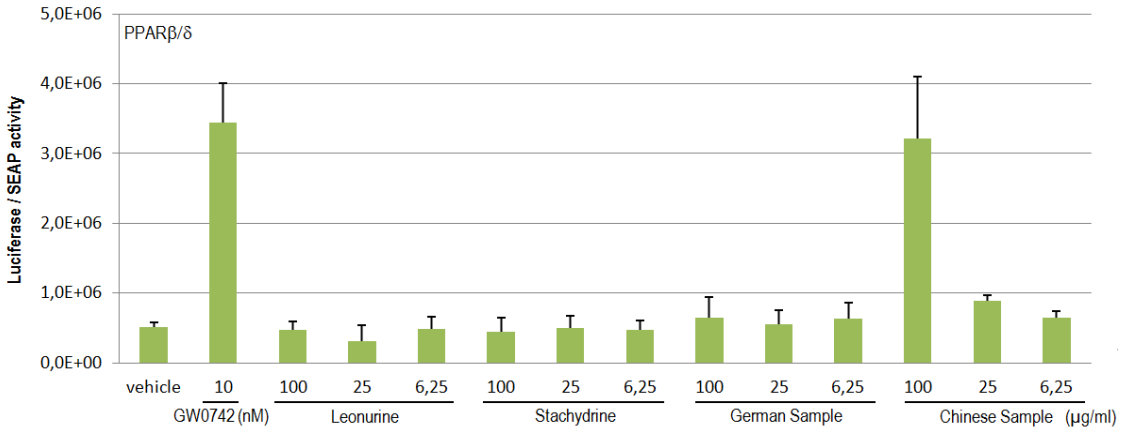
[7] Kuchta K, Ortwein J, Rauwald HW (2012) Leonurus japonicus, Leonurus cardiaca, Leonotis leonurus: A novel HPLC study on the occurrence and content of the pharmacologically active guanidino derivative leonurine. Pharmazie 67: 973-979.

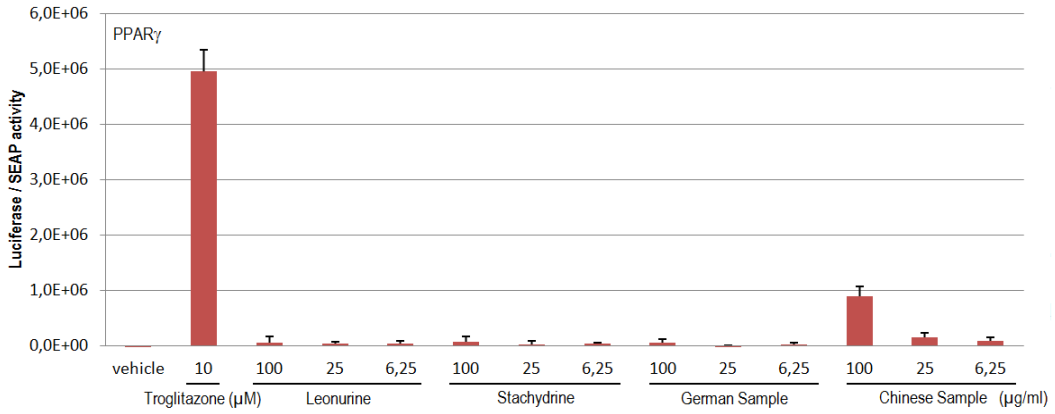
[8] Kuchta K, Ortwein J, Hennig L, Rauwald HW (2014) 1H-qNMR for Direct Quantification of Stachydrine in Leonurus japonicus and L. cardiaca. Fitoterapia 96: 8-17.

(PPAR) α, ß/δ, and γ was investigated using a novel luciferase reporter gene assay. COS-1 cells were co-transfected with the luciferase reporter plasmid p17m2G, containing a GAL4 upstream activating sequence in the promoter region, an expression vector for the human PPARß/δ ligand-binding domain fused to the GAL4 DNA-binding domain pPPARß/δ-GAL4, and the secreted alkaline phosphatase control vector pSEAP. The results are relative to the luciferase expression levels, which were normalized using secreted alkaline phosphatase (SEAP) activity. The two *L. japonicus* extracts and the two isolated constituents (all at 6.25, 25, and 100 µg/ml), and GW0742 (positive control, 0.1 nM) were dissolved in DMSO and added to the medium of the transfected cells. The same approach was used for PPARα and PPARγ in which case the COS-1 cells were transfected with pPPARα or γ-GAL4 and p17m2G, respectively (positive controls 50 µM WY14643 / 10 µM troglitazone). Whereas significant activity was measured for the Chinese Yimucao extract in all three PPAR assays at both 100 and to a lesser extent 25 µg/ml, no activity above DMSO vehicle negative control levels could be detected for the sample from German cultivation or for the two N-containing compounds. Thus, the agonistic activity of Chinese Yimucao in all three PPARs indicates its potential for diabetes and metabolic syndrome therapy. However, the still unidentified active constituents seem to be absent from the German cultivated drug.







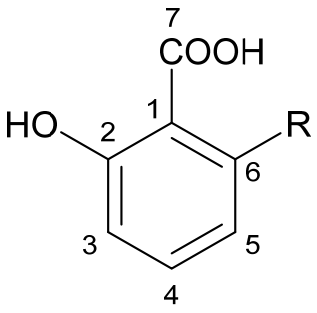


Ginkgolic acids in *Ginkgo biloba* L. leaf TCM extracts: Simultaneous HPLC quantification of all five derivatives using ginkgoneolic acid as a single marker compound [9]

A new HPLC quantification method for allergenic ginkgolic acids (GA) in *Ginkgo biloba* leaf extracts (GBE) was developed, using an acetonitrile-water gradient elution system with an Agilent-SB C18 column. With ginkgoneolic acid (13:0 GA) as a marker, the relative correlation factors of the four other GAs - 15:0, 15:1, 17:1, and 17:2 GA – to 13:0 GA were determined by HPLC and subsequently used for calculating their contents in 10 *G. biloba* leaf samples from Shandong, which were extracted according to the Chin.Ph. monograph for TCM GBE (not identical to the corresponding Ph.Eur. monograph). In other words, the content of 13:0 GA in the

[9] Ruwei Wang, Yuta Kobayashi, Yu Lin, Hans Wilhelm Rauwald, Jianbiao Yao, Ling Fang, Hongxiang Qiao, Kenny Kuchta: HPLC Quantification of All Five Ginkgolic Acid Derivatives in Ginkgo biloba Extracts using 13:0 Ginkgolic Acid as a Single Marker Compound. Planta Med 81: 71-78, 2015.

extracts was determined using the pure compound as an external standard. Subsequently the now known concentration of this compound functioned as an internal standard for the quantification of the other four GA derivatives via the relative correlation factors. The new HPLC method was validated by control measurements with external standards for each individual GA. The results did not differ significantly for any of the 10 leaf samples. Additionally, 10 commercial GBE (9 Chin.Ph., 1 Ph.Eur.) preparations were tested with the new method, in comparison to the current Chin.Ph. protocol. Although all 9 TCM extracts were in accordance with the regulation of a maximal content of 10 ppm GA when tested with the Chin.Ph. HPLC method, only two of these were below this concentration when tested with the new protocol (2.41 vs 9.76 ppm / 2.49 vs 9.52 ppm), demonstrating that in many cases TCM GBE preparations on the Chinese market contain considerable amounts of GA that are not detected by the present Chin.Ph. HPLC. For the Ph.Eur. extract, 0.81 vs 4.87 ppm were measured, both in agreement with the maximal content of 5 ppm GA according to Ph.Eur.. The newly developed protocol is therefore simple, reproducible, and can be used to determine the total contents of GA derivatives in *G. biloba* leaf extracts.



1 C13:0 R = $C_{13}H_{27}$

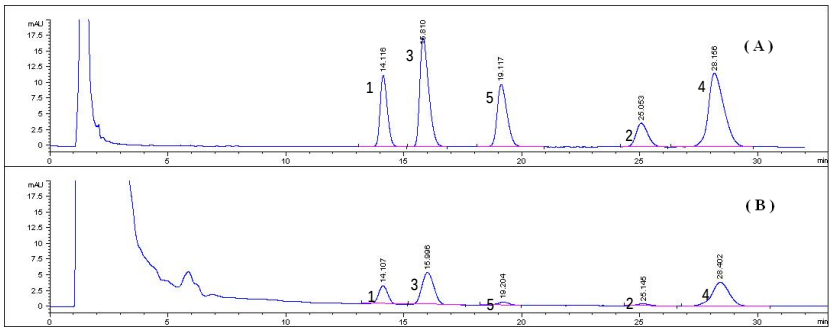
2 C15:0 R = $C_{15}H_{31}$

3 C15:1 R = $C_7H_{14}CH{=}CHC_6H_{13}$

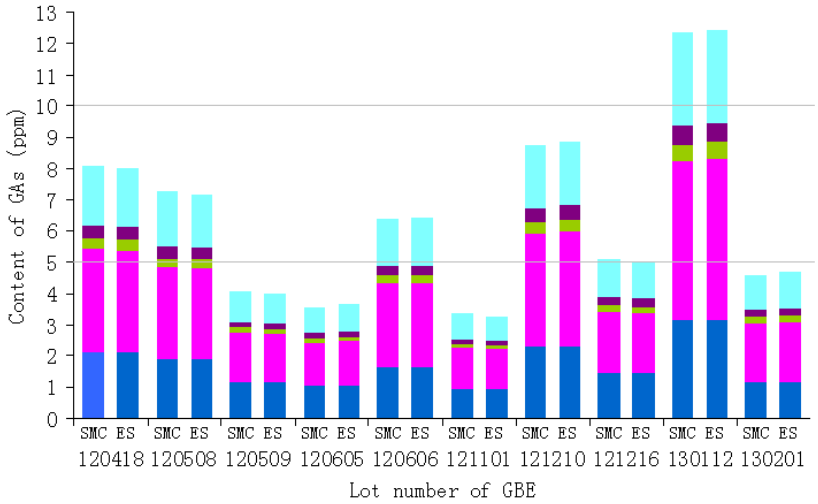
4 C17:1 R = $C_9H_{18}CH{=}CHC_6H_{13}$

5 C17:2 R = $C_8H_{16}CH{=}CHCH_2CH{=}CHC_4H_9$

The five tested ginkgolic acids (1 was also used as marker compound) which are also known by the trivial names ginkgoneolic acid (C13:0) (1), hydroginkgolic acid (C15:0) (2), ginkgolic acid I (C15:1) (3), ginkgolic acid II (C17:1) (4), and anacardic acid c (C17:2) (5). All double bonds in Z configuration.



Representative chromatograms of both the mixed reference standards solution (A) and a typical GBE test sample (lot No. 120418) (B), using the most favourable Agilent SB-C18 column with the respective Agilent 1260 instrument and an Agilent SB C18 (150 mm × 4.6 mm, 5 µm) column (column temperature: 30°C) as well as a gradient elution system with a 0.1% solution of trifluoroacetic acid (TFA) in acetonitrile as component A and a 0.1% solution of TFA in water as component B. The flow rate was kept constant at 1 mL min-1 while the gradient developed over time as follows: t = 0 min: A = 75%; B = 25% / t = 30 min: A = 90%; B = 10% / t = 35 min; A = 90%; B = 10%. The injection volume was set at 50 µL and the detection wavelength at 210 nm. The peaks of the individual tested GA constituents (C13:0) (1), (C15:1) (3), (C17:2) (5), (C15:0) (2), (C17:1) (4) are marked by the respective numbers.



Contents of GAs in the examined GBE samples, measured via individual external standards (ES), and by using 13:0 ginkgolic acid (C13:0) as a single marker compound (SMC). ● C17:1, ● C15:0, ● C17:2, ● C15:1, and ● C13:0. The upper line represents the limit of 10 ppm GAs according to Chin.Ph. while the lower line represents the limit of 5 ppm GAs according to Ph.Eur..

Ginkgo biloba L. leaves and fruits