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The historical evolution of the medicinal use of rosemary (*Rosmarinus officinalis* L.), a Spanish panacea

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The comparison between historical and modern ethnopharmacological works provides interesting insights on the dynamics and evolution of medicinal plant use. This information can be very helpful for understanding why people use and select medicinal plants, and for inferring the future trends of herbal medicine. Using *Rosmarinus officinalis* L. as an example, primary and secondary historical and modern ethnopharmacological sources were searched for the species therapeutic and symbolic uses. Modern phytotherapeutic uses are based on the species' content of essential oil and include internal uses for gastrointestinal problems and external ones for rheumatoid illnesses and circulatory problems (Wichtl 2002). Since Roman and Greek times, rosemary had a prominent devotional significance, whereas its role in medicine was not so prominent. It was both a symbol of love and death. It was used in wedding ceremonies and in the cult of Aphrodite/Venus, the goddess of love. It seems to have played a role in ancient Egyptian death cults, as indicated by a twig of rosemary found in an Egyptian tomb. In ancient Rome, rosemary and olive branches were burned when the body was cremated. One of its ancient names, *libanotis coronaria* ('crown incense'), probably derives from its use as a kind of incense (De Cleene & Lejeune 2003). This highly relevant symbolic plant was later associated with Christian imaginary, and legends appeared linking it to the Virgin Mary. It is likely that the phonetic similarity between 'Mary' and '*marinus'* and its derivates reinforced this link, as indicated by local names such as the German *Mariareinigung*, 'Mary's Purification'. Certainly, its religious associations and magic virtues (used to protect from hailstorms for instance) contributed to the popularisation of rosemary as a medicinal plant. As a medicinal plant, it has been historically employed to relieve tiredness, to cure jaundice, against colds, coughs and other "cold" diseases and its smoke was used against the plague. The renaissance herbals recommend it for all kind of illnesses: as a *digestif* and carminative, to heal wounds, to move benumbed joints, or extremities, for coughs and other respiratory disorders, as a tonic, against weakness, as a blood purifier, to enhance memory, to recover speech, to enhance and procure a clear sight, to freshen breath, against toothache, pest and to expel pestilence. In Spain, it is one of the most popular medicinal plants (Pardo-de-Santayana & Morales 2005) and it is usually collected from the wild. In northern regions, although it does not grow wild, it is a regular element of home gardens. Rosemary oil and alcoholic tincture are very popular in Spain to relieve many kinds of pain, including rheumatic and traumatic muscular pain and pain of the bones. It is also widely used for gastrointestinal and respiratory disorders and many people consider it to be a panacea that cures nearly everything, from alopecia to anxiety or sexual dysfunctions. While today we consider rosemary to be an element of (rational) phytotherapy, this historico-pharmacognostical analysis highlights the importance of the species' symbolic roles in its popularisation as a medicines over centuries.

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A comparative assessment of the processing methods for Radix Angelicae Sinensis (Danggui)

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Radix Angelicae Sinensis (Danggui, DG), the roots of *Angelica sinensis* (Oliv.) Diels, are processed after harvest collection before they are used as Chinese medicinal materials (CMM) according to the traditional experience. The medicinal plants are now grown in 3 major provinces in China. It is one of the most commonly used CMM in Chinese herbal prescriptions for treatment in traditional Chinese medicine. Due to variations in processing procedures of DG used in different cultivation areas in China, the identities and contents of chemical compounds may vary among these DG materials. To develop a standard method for processing DG based on Good Agricultural Practice (GAP) Guidelines stipulated since June 2002 in China, a comparative study was carried out to assess the currently used processing methods. Fresh DG roots were collected from the DG experimental field in Minxian County, Gansu Province of China and processed in different ways. These samples were designated as A, B and C that were fumigated by fumes from burning wheat stems, broad bean stems and wood, respectively; while samples designated as D and E were dried in shade and sunlight, respectively. The five types of processed DG samples were then quantified for their known active ingredients. These were polysaccharides by UV/VIS spectrophotometry, Z-ligustilide, free and total ferulic acid by high-performance liquid chromatography (Lu et al 2004a, 2005). The samples were further analyzed by assessing the developed one and two-dimensional Fourier-transform infrared spectra (FTIR) (Lu et al 2004b). The results (Table 1) showed that the contents of ligustilide, free ferulic acid and total ferulic acid in sample A were highest among the five types of DG samples whereas the level of polysaccha-

Table 1 Bioactive components in Radix Angelica Sinensis processed by different methods

Method	1	2	3	4
A	41.7 ± 0.3	11.61 ± 0.23	0.628 ± 0.01	0.877 ± 0.022
B	36.9 ± 0.5	8.40 ± 0.02	0.573 ± 0.01	0.726 ± 0.008
C	41.7 ± 0.1	9.50 ± 0.12	0.422 ± 0.01	0.702 ± 0.007
D	38.4 ± 1.1	9.85 ± 0.05	0.465 ± 0.01	0.826 ± 0.005
E	46.9 ± 1.3	10.86 ± 0.11	0.480 ± 0.01	0.875 ± 0.012

1, Polysaccharides; 2, Z-ligustilide; 3, Free ferulic acid;
4, Total ferulic acid. Data are means ± s.d., n = 4, mg/g.

rides in sample A was also relatively higher in content. Comparing the 2D-FTIR spectra, it was found that samples A and B, C and D were similar to one another but different from sample E. The overall results indicate that the quality of sample A is relatively the best among the others, implying that the processing method of fumigation from burning wheat stems can be considered as the most suitable for processing DG, as reflected by the analytical data obtained for some of the active ingredients.

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Screening of indigenous medicinal plants for anthelmintic activity

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In Kenya, nomadic pastoralists popularly use herbal medicines for the treatment of human and animal diseases. These communities live in arid and semi-arid lands rich in medicinal plants. Of the diseases affecting livestock, helminthiasis is reported to account for more than one-third of all the diseases that affect sheep and goats in Kenya. The economic loss due to helminthes infestation in goats alone is estimated at US$ 26 million (Barbara et al 1991). Conventional anthelmintic drugs are expensive, have side effects, and parasites soon develop resistance on continued use. Hence there is need to search for other alternative remedies. Most of the research on herbal anthelmintics has concentrated on in-vivo tests using infected animals (Abebe et al 2000). Faecal matter from drenched animals was analysed for helminthes eggs burden to determine the efficacy of the herbal drugs. Recently, Wasswa et al (2006) reported in-vitro ascaricidal activity of selected medicinal plants endemic to Uganda. The objective of the present study was to screen medicinal plants purported to have anthelmintic activity in some parts of Eastern Province, Kenya. Ten plants with purported anthelmintic activity were collected and authenticated by a plant taxonomist from the East African Herbarium. The medicinal plant parts were dried under a shade, and ground to a fine powder. Aqueous extracts were prepared (5 g of powder into 50 ml of boiled distilled water). Helminthes larvae identified as a mixture of *Ancyclostoma doudenali*, *Necator Americana*, and *Strongloides stercolaris* in goat faecal matter were used in this experiment. Ten helminthes larvae at L_1 stage were placed into a sterile petri dish containing 10 ml of Goodwin's physiological solution. Five milliliters of the plant extract was then added and agitated for uniform mixing. The procedure was repeated for Nilzan plus and Wormicid (commercial drugs based on levimisole), which were used as positive controls. Each experiment was done in triplicate. Dead larvae were counted after every 24 h. The percentages of dead larvae were reported for the first two days as follows: 65% day 1, 80% day 2 (Nilzam plus); 65% day 1, 90% day 2 (Wormicid); 20% day 1, 60% day 2 (*Harrisonia abyssinica*); 62% day 1, 90% day2 (*Skhuria pinnata)*; 90% day 1, 00% day 2 (*Albizia anthelmintica*); 20% day 1, 80% day 2 (*Fagalopsis angolensis)*; 90% day 1, 100% day 2 *(Entada leptostachya*); 60% day1, 100% day2 *(Vernonia lasiopus)*; 40% day 1, 60% day 2 (*Senna singuana*); 80% day1, 80% day 2 (*Clerodendrum eriophylla)*; 58% day 1, 100% day 2 (*Warburgia ugandensis*). The results indicate that the selected plants had anthelmintic activity . Four plant extracts killed all the larvae after 48 h.

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Antioxidant activity and fingerprinting of Spanish *Bupleurum* species used as anti-inflammatory remedies

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Bupleurum species (Umbelliferae) turned out to be promising sources of potent anti-inflammatory compounds (Bremner et al 2004; Prieto et al 2004). The dried aerial parts of *Bupleurum fruticescens* Loefl. subsp. *fruticescens* (BF) and *Bupleurum rigidum* L. subsp. *rigidum* (BR) were collected near Madrid (Spain), identified, and conditioned by one of the authors (MPS) and voucher specimens are deposited in our institution. Different extracts of this material were tested for their antioxidant activity by using the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and lipid peroxidation in bovine brain liposomes (LPBBL) following the protocols described by Burits & Bucar (2000). Both *Bupleurum* species were processed in same way. The crude or total extract was obtained by macerating in methanol. Extracts containing compounds of increased polarity were obtained by extracting the same material sequentially with *n*-hexane (Hex), ethyl acetate (EtOAc) and methanol (MeOH). The IC_{50} of each extract in the antioxidant assays are reported in Table 1. Trolox was used as reference and its IC_{50} were 25 and 32 μM in the DDPH and LPBBL assays, respectively. In general the two *Bupleurum* species showed different antioxidant profiles, *B. rigidum* being more active as scavenger of DPPH and *B. fruticescens* more active as inhibitor of the lipid peroxidation. The antioxidant activity of the extracts in the DPPH model was positively correlated with their content in total phenols measured by the Folin-Ciocalteau method. TLC analysis of the extracts (silica gel; ethyl acetate/acetic acid/water 65:15:20 followed by spraying with 1% diphenylboric acid 2-aminoethyl ester then 5% polyethylene glycol and examination under UV light 365 nm or with DPPH – 0.04% methanolic solution) showed that the DPPH scavenging activity was mainly due to the presence of flavonoids and other phenols. The HPLC-UV analyses of the crude, EtOAc and MeOH extracts showed that both species have different fingerprints. The main difference was the presence of rutin as a major compound in *B. fruticescens* and its absence in *B. rigidum*.

Table 1 IC_{50} of the *Bupleurum* extracts

	DPPH		LPBBL	
	BF	BR	BF	BR
Crude	0.179	0.135	0.328	0.910
Hex	2.367	0.909	0.347	1.699
EtOAc	0.236	0.536	0.108	0.646
MeOH	0.187	0.085	0.594	0.903

The abbreviations are given in the text.

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Catechins from betel nut contribute to its overall cholinergic effect

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The shredded seeds of *Areca catechu* L. (Palmaceae), commonly known as betel nut, are used as a masticatory by over 500 million people in the world. A variety of preparations are used with either dried or roasted seeds which can be mixed with lime (crude calcium hydroxide), and sometimes dried catechu, rolled together in a leaf of *Piper betel*. Its popularity is thought to be due to the mild stimulant effect on the CNS, caused by the cholinergic activity of arecoline, the major alkaloid present in *A. catechu*. Recent work has shown that the extract also inhibits acetylcholinesterase, and this would further enhance the cholinergic effect by elevating levels of acetylcholine (ACh) in the CNS (Gilani et al 2004) but the nature of the compounds responsible has not been elucidated. Samples of various commercial brands of *A. catechu* seeds were obtained from retail outlets and from the museum of the Dept of Pharmacy KCL. They were sequentially extracted with hexane, chloroform and ethyl acetate and examined by double development TLC (silica gel GF_{254}/ethyl acetate:methanol 4:1 for 5 cm, dried and then chloroform:methanol 8:1 for 15 cm). Plates were examined under UV light 254 nm and then either sprayed with Dragendorff's reagent, 5% aq. $Fe(III)Cl_3$ or for AChE inhibitory activity using the Ellman rection (Rhee et al 2001, 2003). Although the intensities of the various zones varied between different samples, all gave a similar pattern of zones. Zones giving a false positive reaction for AChE inhibitory activity (Rhee et al 2003) were seen with the hexane extract, but the ethyl acetate extract gave two prominent zones with a true effect. No inhibitory zone was seen in the chloroform extract, which, however, gave a major Dragendorff-positive zone corresponding to the reference compound arecoline. The AChE inhibitory zones in the ethyl acetate extract gave a blue-green colour with the 5% aq. $Fe(III)Cl_3$ reagent and were found to be identical with (+)-catechin and (−)-epicatechin. A range of concentrations of these two compounds was tested for AChE inhibitory activity using the Ellman reaction and both were found to be fairly strong AChE inhibitors with IC_{50} 12.54 ± 1.34 μM for (+)-cate-

chin and 6.12 ± 0.78 μM for (−)-epicatechin. If these compounds are absorbed into the blood following mastication of betel nut, then they are likely to reach the CNS and peripheral system, and complement the cholinergic activity of the arecoline present in the betel nut by producing an overall rise in ACh levels.

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Cytotoxic stilbenes from *Cajanus Cajan* (L.) Millsp. leaves

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Cancer remains one of the leading causes of death in the world especially in developed countries and the search for new therapeutic agents continues. Since plants have yielded a number of therapeutically useful anticancer compounds, the screening for cytotoxicity of plants used traditionally to treat cancer is rational. *Cajanus cajan* (Fabaceae), commonly called Pigeon pea, is grown for food and medicinal purposes in the tropics, especially Nigeria. An extract has been reported to display significant cytotoxic activity (Ashidi et al 2005). We now report a biologically monitored phytochemical separation of the leaves of *Cajanus cajan* against a panel of human cancer and non-cancer cell lines in vitro. The air dried leaves were extracted with methanol continuously for five days. The extract was then concentrated under reduced pressure. 60 g of the extract was adsorbed on Silica Gel GF_{254} and separated into fractions by vacuum liquid chromatography (VLC). The SRB assay was used to evaluate the cytotoxicity of the extracts and the isolated compounds. The dichloromethane (DCM) fraction of the leaves exhibited modest cytotoxicity against human amelanotic melanoma — C32, human breast adenocarcinoma — MCF-7 and human large cell lung carcinoma cell lines — COR-L23 and human fetal lung fibroblast — MRC-5 (IC_{50} = 12.0, 10.0, 10.0 and 15.0 μg/ml, respectively). Vinblastine sulphate (Sigma) was used as positive control. This finding prompted further activity-guided fractionation of the DCM fraction by flash chromatography and subsequent purification on preparative thin-layer chromatography, which led to the identification of two prenylated stilbenes, longistylin A and C. The structures of the compounds were determined by both ^{1}H and ^{13}C NMR and Mass spectroscopy. The ^{13}C NMR spectra agreed with previously published data (Ashidi et al 2005). These compounds have previously been reported to have antiplasmodial activity(Duker-Eshun et al 2004). This is also the first time that these prenylated stilbenes are shown to exhibit in-vitro cytotoxic activity against human amelanotic melanoma, C32, human breast adenocarcinoma, MCF-7, and human large cell lung carcinoma, COR-L23, cell lines. The IC_{50} of the compounds ranges between 20 and 35 μM. These compounds could explain the rational inclusion of *Cajanus cajan* in traditional herbal medicines used for the treatment of cancer in south- western Nigeria. Further study to establish the mechanism of action of these two stilbenes is in progress.

Acknowledgement: JSA thanks the Association of Commonwealth Universities, UK, for financial support.

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CYP enzyme inhibition by Echinacea and its alkamides: is it clinically relevant?

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Echinacea preparations are one of the best selling herbal medicinal products (HMPs) with a well-established therapeutic use in the treatment of upper respiratory tract infections and other minor conditions. Their use is increasing, but information regarding possible interactions with other medicines is scarce. Many of these interactions occur via induction or inhibition of the CYP P450 enzyme system. The cytochrome P450s are a superfamily of membrane-bound, haeme-thiolate proteins, which are principally responsible for phase 1 oxidative metabolism and detoxification of xenobiotics and endobiotics (e.g. steroids, fatty acids, retinoids, eicosanoids, vitamins, cholesterol and prostaglandins). The objective was to analyse the inhibitory potential of the standardised Echinacea extract (Echinaforce) and two alkamides: dodeca 2E,4E,8Z,10E/Z tetranoic acid isobutylamide (TAI) and dodeca 2E,4E-dienoic acid isobutylamide (DAI) on single baculovirus expressed Cytochrome P450 isoforms – CYP1A2, CYP2C19, CYP2D6 and CYP3A4 as stipulated by the German regulatory authority BfArM (Bundesinstitut fur Arzneimittel und Medizinprodukte) (BfArM website). In a modified fluorometric 96-well plate assay enzyme activity was measured by detecting the fluorescent metabolite produced from the reaction of the substrate with the CYPs (Crespi 1997). The substrates used were7-benzyl-4-(trifluoromethyl)-coumarin (BFC) (CYP3A4), 3-Cyano-7-Ethoxy-Coumarin (CEC) (CYP1A2, CYP2C19) and 3-(2-N, N-diethyl-N-methylaminoethyl)-7-methoxy-4-methylcoumarin (AMMC) (CYP2D6). Control reactions were also set up to account for intrinsic fluorescence of the extract and the effect of ethanol on the enzyme. Both the extract and the individual alkamides showed moderate inhibitory activity against CYP enzymes, but these effects are unlikely at the doses of Echinaforce normally encountered in clinical setting (Table 1). The inhibitory potency of the extract cannot be explained entirely in terms of its alkamide content, particularly since both alkamides showed no inhibition of CYP1A2, which is nevertheless inhibited by the extract. The lowest IC50 value recorded in our study was 1.96μg/ml for TAI. Based upon a recent bioavailability study, these values would be 4900 folds higher than the anticipated maximal concentration in hepatocytes, assuming that there are no potential loses of the alkamide via distribution, uptake etc (Bauer 2005). With these IC_{50} values it is unlikely that inhibitory concentrations will be reached within the liver.

Table 1 Median inhibitory concentrations (IC_{50}) and the upper and lower 95% confidence limits (depicted in brackets) for Echinaforce, dienoic acid isobutylamide and tetranoic acid isobutylamide against the CYP isoforms – CYP1A2, CYP2C19, CYP2D6 and CYP3A4

Test substance	CYP1A2	CYP2C19	CYP2D6	CYP3A4
Echinaforce (μg/ml)	26.54 (23.81–29.57)	53.47 (32.30–88.79) 7	60.97 (50.29–3.91)	19.49 (18.80–20.20)
DAI (μg/ml)	No inhibition	23.35 (17.37–31.40)	10.10 (7.209–14.151)	5.17 (4.11–6.52)
TAI (μg/ml)	No inhibition	18.91 (14.86–24.05)	6.76 (5.21–8.77)	1.91 (1.74–2.09)

Funding: This project is sponsored by Bioforce, UK

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Variation in content and antifungal activity in samples of asafetida

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Asafoetida is a resinous substances with a smell similar to garlic, which is obtained by drying the exudates from various species of *Ferula* growing in northern Iraq and Iran and surrounding countries. It is widely used in cooking in India and is used medicinally for gastro-intestinal complaints and for treating skin diseases. The botanical source of commercial samples of asafoetida is not easy to determine since several species of *Ferula* exist. Samples of asafetida with proven source were obtained from the pharmacognosy museum of King's College London and compared with some commercial samples obtained from Asian shops in the UK, India and Syria. Samples were examined by TLC (silica gel GF_{254}/toluene:methanol 95:5) visualizing under UV light 254 nm and 365 nm, then in daylight after spraying with acidic anisaldehyde and heating for 10 min at 105°. The samples displayed considerable variation in their chemical profile with the Indian variety being much more similar to commercial mixtures of flour and asafetida sold in the UK for use in cooking. The various samples were tested for antifungal activity using the serial dilution method (Mensah et al 2000) against two dermatophytes, *Microsporeum gypseum* and *Trichophyton interdigitale*, using miconazole as a positive control. The most active sample was a resin obtained from Syria and conformed most closely to a museum sample from *F. foetida*. From this sample nine prenylated coumarins were isolated using preparative HPLC and the structures determined by advanced spectroscopic methods including heteronuclear NMR. All compounds isolated were tested against the two fungal species. Four of the compounds exhibited strong antifungal activity against the dermatophytes and these were farnesylferol, an isomer of farnesylferol, galbanic acid and 5,8 dihydroxyumbelliprenin (Table 1). 5,8 Dihydroxyumbelliprenin was the most active with MIC of 10 mM, the positive con-

Table 1 Compounds from asafetida and their antidermatophytic activity at 10 μL of 10 mM solution (zone of inhibition in mm) n = 3

Compound	*M. gypseum*	*T. interdigitale*
Isomer of farnesylferol C	5	5
Galbanic acid	13	12
Kataravicinol	0	0
5,8-dihydroxy umbelliprenin	26	24
Umbelliprenin	0	0
Farnesylferol C	16	15
Umbelliferone	0	0
3-O-acetylepismarcadin	0	0
Foetidine	0	0
Miconazole	23	22

trol miconazole having MIC of 0.5 mM. No compounds of this type have previously been shown to have antifungal activity. Two of these compounds were also found in another active sample, a commercial oleoresin extracted from asafoetida obtained from India.

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Local Mediterranean food as a source of novel nutraceuticals

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There are still many rural regions and communities in Southern Europe, which follow traditional lifestyles. Food is an example exemplifying local knowledge and the use of local food species potentially has considerable health benefits. The Mediterranean traditional food knowledge has sometimes been described as a diet mainly composed by pasta, olive oil, vegetables, fruits, red wine, sea-foods and only few red meats. A hitherto little-studied aspect is locally collected wild greens. This project focused specifically on new sources for natural and nutritional antioxidants and included a *primary screening of wild food plants* collected in Southern Italy, South Eastern Spain and Greece. Here we only focus on the plants collected in the Graecanic (Greek speaking) community of Gallicanó in Southern Italy (Heinrich et al 2005; Nebel 2005 Nebel et al 2006). A large panel of primary screening assays were used by the partners of the consortium 'Local Food-Nutraceuticals' to select species with the highest in vitro activity. At our institution an antioxidant assay (measurement of free radical scavenging activity (FRSA) in the 1,1-diphenyl-2-picrylhydrazil radical (DPPH) assay) and the inhibition of the xanthine oxidase were employed for the primary screening. *Reichardia picroides* Roth (Asteraceae) collected both from Gallicanó and Crete (Greece) showed the highest overall activity

Table 1 DPPH scavenging activity and inhibition of xanthine oxidase of wild food plants

Plant species	Xanthine Oxidase Inhibition in % (100 μg/ml)		DPPH-scavenging activity in % (1 mg/ml)		Polyphenol content (mg/g)
	n = 3	STD	n = 3	STD	
Reichardia picroides	22.1	6.0	32.2	3.2	335.12
Papaver rhoeas	21.4	7.6	16.5	11.4	34.14
Carlina acaulis	48.3	2.1	22	5.6	59.1
Sonchus oleraceus	12.1	6.7	42.6	4.9	157.2
Urospermum dalechampii	28.3	7.9	26.2	4.5	80.6
Chondrilla juncea	8.3	6.6	30.5	5.4	117.8
Quercetin 50*/100[&]MMol	47.8*	3.6	24.0[&]	1.9	—

score of the species from this region (Table 1). *Papaver rhoeas* L. (Papaveraceae), a species also collected in other regions of Italy and in Greece also showed noteworthy activity (The Local Food-Nutraceuticals Consortium 2005). The chemical composition of *Reseda alba* is practically unknown. Only one compound, 2-hydroxy-2-methyl-propyl-glucosinolate, had previously been isolated (Gmelin & Kjaer 1970). In the primary screen it showed noteworthy activity in the measurement of lipid peroxidation in mouse brain tissue (MDA, The Local Food-Nutraceuticals Consortium 2005). The dried plant material (890 g dried young leaves) ground and extracted with dichloromethane and methanol (cold percolation) yielded in 18 g dichloromethane extract and 225 g methanol extract, respectively. Flavonoid-type compounds were isolated using preparative thin layer chromatography (PTLC) and the purity of the samples was verified with TLC and ^{1}H NMR. The structure was elucidated using Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). From the methanol extract five flavonoid glycosides were isolated. One of the isolated flavonoid was identified as kaempferol-3,7-O-α-L-dirhamnoside.

We gratefully acknowledge the input of all members of the consortium 'Local Food-Nutraceuticals' co-ordinated by MH (see http://www.biozentrum.uni-frankfurt.de/Pharmakologie/EU-Web/index.html) and funding by the EU (QLRT-2001–00173; 2002 – 2004).

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