Forgotten Edible Alpine Plants in the Canton of Valais

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Dekan

To my fiancée,

Alexandra

and my family

« La vraie nouveauté naît toujours dans le retour aux sources. »

Edgard Morin

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List of abbreviations

BAG	Bundesamt für Gesundheit
BMI	Body Mass Index
DAD	Diode ArrayDetector
DASH	Dietary Approaches to Stop Hypertension
FID	Flame Ionization Detector
GC	Gas Chromatography
GDP	Gross Domestic Product
HPLC	High Performance Liquid Chromatography
LDL	Low Density Lipoprotein
MAPs	Medicinal and Aromatic Plants
TCM	Traditional Chinese Medicine
TLC	Thin Layer Chromatography
TOF-MS	Time of Flight Mass Spectrometry
USDA	United States Department of Agriculture
WHO	World Health Organization

Summary

The Swiss Health Office encourages citizen to eat more fruits and vegetables, which have shown beneficial properties on health and contain amounts of nutrients and substances essential for the organism. The strong connection between nutrition and health is increasingly recognized. Proposing new kinds of fruits and vegetables with a high content in beneficial substances and with attractive gustatory properties could be a way forward to achieve the goals of the Swiss Health Office.

Tradition possesses plenty of forgotten wild edible plants and may help researchers in the quest for new food varieties. Swiss alpine cantons, especially the canton of Valais, have still had a viable tradition. However, societal changes and extensive urbanization have caused this knowledge to be confined to lateral valleys. This contribution aimed to document wild edible plants which were collected in the canton of Valais. 38 informants originating from four different valleys of the canton (Val d'Entremont, Val d'Illiez, Val d'Hérens, and Val d'Anniviers) were interviewed with semi-directive interviews.

98 edible plant species, which belong to 38 families, were identified. Plants were classified in eight categories based on the way they were traditionally used including salads, cooked vegetables, spices, alcoholic drinks, teas, syrups, jams, and raw snacks. The categories with the highest number of citations were teas (18%), followed by cooked vegetables (16%), jams (16%), and raw snacks (16%). *Taraxacum officinale*, *Sambucus nigra*, *Chenopodium bonus-henricus*, and *Urtica dioica* were the most cited plants and most commonly used in the different valleys. Knowledge on edible plants is found from its origins in agriculture and activities as shepherds. Books written in the XIXth and early XXth centuries have documented these uses and have allowed identification of around 40 food plants, which had already fallen in oblivion (e.g. *Bunium bulbocastanum*).

Two edible plants issued from the Valais tradition (*Phyteuma orbiculare* and *Cirsium spinosissimum*) were submitted to a thorough phytochemical investigation. Each plant was successively extracted with dichloromethane and methanol. Extracts were subjected to HPLC-MS DAD analyses and pure constituents were isolated by preparative and semi-preparative methods (Diaion HP-20, liquid-liquid extraction, Sephadex LH-20, open column on silica gel,

preparative and semi-preparative columns on C18). The molecular structures of the isolated compounds were elucidated by chemical and spectroscopic methods. In addition, substances relevant for nutrition (e.g. vitamins, fatty acids, minerals, and major polyphenols) were quantified.

The first species investigated was the round-headed rampion (*Phyteuma orbiculare* L., Campanulaceae). The sweet flowers of the plant were consumed by shepherds as raw snacks, whereas nutty-tasting leaves (rosettes) were eaten as a salad. No phytochemical studies or biological data had been published for the entire genus *Phyteuma*.

23 substances including different polyphenols, fatty acids, and triterpenes were identified from dried aerial parts. Phytochemical investigations also revealed the presence of two novel saponins, phyteumosides A and B. The aglycon of phyteumoside A possessed an unprecedented skeleton that could be rationalized as an incompletely cyclized onoceroid triterpene, whereas that of phyteumoside B was a new 17-polypodene skeleton. Identification of these two substances was achieved by compilation of chemical and enzymatic hydrolyses, followed MS/MS, GC-MS, NMR and X-rays analyses. In addition to these two new substances, a new dimeric phenylpropanoid glycosylate derivative (tangshenoside VII) could be isolated and elucidated.

Concerning the quantification of substances relevant for nutrition, *Phyteuma orbiculare* contained interesting amounts of ascorbic acid, β-carotene, polyphenols, polyinsaturated fatty acids, calcium, magnesium and potassium

This food plant, which possesses interesting nutritive properties and favorable breeding predispositions, could be an interesting candidate for further agronomic development. However, species of the same genus have a larger biomass and it was interesting to compare their phytochemical profile. HPLC-MS DAD analyses revealed similar metabolite profiles for *P. spicatum*, *P. ovatum*, and *P. orbiculare* but showed differences for *P. hemisphaericum*.

The second plant to be investigated was a thistle, *Cirsium spinosissimum* (Asteraceae). Surrounding leaves and the pappus hairs were removed before consumption, and the receptacle was eaten in early summer time. Taste of the receptacle is similar to that of an artichoke, and its consistency is tender.

A total of 20 substances including polyphenols, a monoterpene lactone, fatty acids and a spermine derivative were identified. Major polyphenols were linarin and pectolinarin and have been previously isolated from other *Cirsium* species.

This plant contains vitamins and polyunsaturated fatty acids in low amounts, and an interesting level of potassium. *Cirsium spinosissimum* is not really convenient for further cultivation due to its spiny morphology, complex preparation, and a nutritive value in the average range.

Other alpine edible plants selected during this work could be interesting with regard to their chemical composition, and for future breeding. They should be the main focus of further investigations. The establishment of alpine plants as new food crops would represent a diversification of the activities in mountain agriculture.

Zusammenfassung

Das Bundesamt für Gesundheit (BAG) ermutigt die Schweizer Bevölkerung, mehr Obst und Gemüse zu konsumieren. Diese Lebensmittel enthalten zahlreiche Nährstoffe, die für den menschlichen Organismus essentiell sind, und haben vorteilhafte Auswirkungen auf die Gesundheit gezeigt. Die enge Verbindung zwischen Ernährung und Gesundheit wird zunehmend anerkannt. Ein breiteres Angebot von Obst- und Gemüsesorten mit angenehmen gustatorischen Eigenschaften könnte dabei das Erreichen der Ziele des BAG massgebend erleichtern.

Die Tradition ist reich an vergessenen wilden, essbaren Pflanzen und kann für Forschende auf der Suche nach neuen Nahrungssorten eine Inspirationsquelle sein. Schweizer Alpenkantone, darunter insbesondere das Wallis, verfügen seit jeher über eine lebendige Tradition. Allerdings haben gesellschaftliche Veränderungen, extensive Urbanisierung und Pflanzenunkenntnisse zur Begrenzung des Wissens beigetragen.

Diese Dissertation ist eine Zusammenstellung wilder, essbarer Pflanzen aus dem Wallis, die von der Bevölkerung traditionellerweise gesammelt wurden. 38 Personen aus vier verschiedenen Tälern (Val d'Entremont, Val d'Illiez, Val d'Hérens und Val d'Anniviers) wurden dazu in Leitfadeninterviews befragt. 98 essbare Pflanzenarten, die zu 38 Familien gehören, konnten auf diese Weise identifiziert werden. Die Pflanzen wurden in 8 Kategorien eingeteilt, basierend auf deren traditioneller Verwendung (Salate, gekochtes Gemüse, Gewürze, alkoholische Getränke, Tees, Sirupe, Marmeladen und Snacks). Die Kategorien mit der höchsten Zahl der Nennungen waren Tees (18%), gefolgt von gekochtem Gemüse (16%), Marmeladen (16%) und rohe Snacks (16%). Löwenzahn (*Taraxacum officinale*), Holunder (*Sambucus nigra*), Guter Heinrich (*Chenopodium bonus-henricus*) und Brennessel (*Urtica dioica*) waren die am häufigsten angegebenen Pflanzenspezies, die in den verschiedenen Tälerns des Kantons Wallis verwendet wurden.

Die Kenntnisse über essbare Pflanzen finden ihren Ursprung in der Landwirtschaft und in der Tätigkeit als Hirten. Das Lesen von Büchern aus dem 19. und aus dem frühen 20. Jahrhundert ermöglichte in dieser Dissertation die Identifizierung von rund 20 Nahrungspflanzen, die bereits in Vergessenheit geraten sind (z.B. *Bunium bulbocastanum*).

Zwei essbare Pflanzen, die traditionellerweise im Wallis konsumiert wurden (*Phyteuma orbiculare* und *Cirsium spinosissimum*), wurden im Rahmen dieser Doktorarbeit wissenschaftlich vertieft untersucht. Beide Pflanzen wurden stufenweise mit Dichlormethan und Methanol extrahiert. Die Extrakte wurden mit Hilfe von HPLC-MS DAD analysiert und Reinsubstanzen wurden mittels präparativer und semi-präparativer HPLC isoliert (Diaion HP-20, Flüssig-Flüssig-Extraktion, Sephadex LH-20, offene Säule mit Silicagel, präparative und semi-präparative HPLC mit C18-Säulen). Die molekularen Strukturen der isolierten Verbindungen wurden mittels chemischer und spektroskopischer Analysen aufgeklärt. Darüber hinaus wurden für die Ernährung relevante Substanzen (z.B. Vitamine, mehrfach ungesässtigte Fettsäuren, Mineralien und Polyphenole) quantifiziert.

Die erste zu untersuchende Spezies im Zuge dieser Dissertation war die kugelige Teufelskralle (*Phyteuma orbiculare* L., Campanulaceae). Die süssen Blüten der Pflanze wurden angeblich von Hirten als Snacks verzehrt, während die nussig schmeckenden Blätter (Rosetten) vorrangig als Salat konsumiert wurden. Über die Spezies oder Gattung von *P. orbiculare* wurden bislang keine phytochemische Studien durchgeführt.

Insgesamt konnten 23 verschiedene Inhaltsstoffe, darunter Polyphenole, Fettsäuren und Triterpene, in den getrockneten oberirdischen Teilen von *P. orbiculare* identifiziert werden. Phytochemische Untersuchungen führten überdies zur Entdeckung von zwei neuen, bislang nicht identifizierten Saponinen, die Phyteumoside A und B benannt wurden. Das Aglykon von Phyteumoside A besitzt ein neuartiges Skelett, das als unvollständig zyklisiertes Onoceroid betrachtet werden kann, dasjenige von Phyteumoside B verfügt über ein neuartiges 17-Polypoden Skelett. Die Identifizierung der beiden Substanzen erfolgte mittels chemischer und enzymatischer Hydrolysen, MS/MS, GC-MS, HPLC-MS TOF, NMR und Röntgenkristallographie. Weiter konnte ein bisher unbekanntes glykosiliertes Phenylpropanderivat (Tangshenoside VII) identifiziert und isoliert werden. Vergleiche zwischen getrockneten und frischen Materialien zeigten ähnliche HPLC-MS DAD Profile. In Bezug auf die Inhaltsstoffe, die für die Ernährung relevant sind, konnten in *P. orbiculare* interessante Mengen an Ascorbinsäure, beta-Carotin, Polyphenolen, mehrfach ungesättigte Fettsäuren, Calcium, Magnesium und Kalium nachgewiesen werden. Aufgrund der interessanten nutritiven Eigenschaften sowie der vorteilhaften Kulturbedingungen von *P. orbiculare*, stellt diese Nahrungspflanze ein vielversprechender Kandidat für künftige agronomische Studien dar. Da zur Gattung *Phyteuma* einige Spezies mit grösserer Biomasse

zählen, wurden zudem Vergleichsanalysen durchgeführt. Mit Ausnahme von *P. hemisphaericum* zeigten *P. spicatum*, *P. ovatum* und *P. nigrum* ähnliche Metabolitenprofile wie *P. orbiculare*.

Die zweite Pflanze, die wissenschaftlich vertieft untersucht wurde, war die Distel *Cirsium spinosissimum* (Asteracea). Die Blütenböden der Pflanze wurden im Frühsommer konsumiert, wobei Haarkranz und umgebende Blätter vor dem Verzehr entfernt wurden. Der Geschmack der Blütenböden ähnelt demjenigen der Artischocke und die Konsistenz ist zart. Insgesamt 20 Substanzen, darunter Polyphenole, Fettsäuren, ein Monoterpen-Lacton und ein Spermin-Derivat, konnten in *C. spinosissimum* identifiziert werden. Hauptpolyphenole waren Linarin und Pectolinarin, die bereits aus anderen *Cirsium*-Artenisoliert wurden. In *C. spinosissimum* konnten Vitamine und eine interessante Menge an Kalium nachgewiesen werden, jedoch waren ungesättigte Fettsäuren nur in einer geringen Menge enthalten. Für die Kultivierung ist die Nahrungspflanze aufgrund ihrer stacheligen Morphologie, der Aufbereitungsschwierigkeit vor dem Verzehr und des nur durchschnittlichen Nährwertes jedoch nicht besonders geeignet.

Andere essbare Alpenpflanzen dagegen, die im Rahmen dieser Dissertation identifiziert wurden, könnten von bedeutendem wissenschaftlichem und agronomischem Interesse sein. Weitere Forschungsarbeiten darüber sind notwendig und würden der Berglandwirtschaft Abwechslung in der Kultivierungsaktivität erlauben.

Résumé

L'Office Fédéral de la Santé Publique encourage la population suisse à consommer davantage de fruits et légumes. Ces aliments ont en effet démontré des effets bénéfiques sur la santé et contiennent de nombreux nutriments essentiels au bon fonctionnement de l'organisme. Il existe des liens établis de plus en plus étroits entre nourriture et santé. Proposer une variété plus grande de fruits et légumes avec des espèces dotées de propriétés gustatives agréables pourrait être envisagé afin d'atteindre le but que s'est fixé l'organisation faitière.

La tradition regorge de plantes sauvages comestibles oubliées et pourrait ainsi inspirer les chercheurs dans leur quête de nouvelles saveurs. Les cantons suisses alpins et en particulier le Valais possèdent toujours une tradition vivante. Toutefois, les changements sociétaux et l'urbanisation croissante fait que ce savoir est de plus en plus confiné dans des vallées latérales du Valais. Ce travail propose ainsi de répertorier les plantes comestibles sauvages que certains Valaisans ramassent. 38 personnes originaires de 4 vallées du Valais (Val d'Entremont, Val d'Illiez, Val d'Anniviers et Val d'Hérens) ont pu être approchées et interrogées au moyen d'interviews semi-directives.

98 espèces végétales comestibles sauvages appartenant à 38 familles ont pu être identifiées. Ces plantes ont été classées selon leur mode de consommation en 8 catégories comprenant les salades, les légumes cuits, les épices, les friandises, les boissons alcoolisées, les thés, les sirops et enfin les confitures. Les plantes sont surtout utilisées sous forme de tisanes (18%), suivies des friandises, des confitures et des légumes cuits qui représentent chacun 16% des modes de consommation. Parmi les plantes souvent citées, le pissenlit (*Taraxacum officinale*), l'ortie (*Urtica dioica*), le sureau (*Sambucus nigra*) et le chénopode bon-henri (*Chenopodium bonus-henricus*) semblent être significativement utilisées dans les différentes vallées du canton. La connaissance sur les plantes alimentaires provient surtout des activités agricoles et en tant que bergers des personnes interviewées. La lecture d'anciens livres datant du XIXe siècle a également permis d'identifier une quarantaine de plantes comestibles tombées en désuétude (p.e *Bunium bulbocastanum*).

Deux espèces consommées dans la tradition valaisanne (*Phyteuma orbiculare* et *Cirsium spinosissimum*) ont ensuite été investiguées scientifiquement. Les plantes ont chacune été

extraites d'abord par du dichlorométhane, puis par du méthanol. Chaque extrait a ensuite été soumis à des analyses HPLC-MS DAD et les constituants ont été isolés grâce à une palette de méthodes préparatives (Diaion HP-20, extraction liquide-liquide, Sephadex LH-20, colonne ouverte sur gel de silice et HPLC semi-préparative et préparative sur C18). Les composés isolés ont été identifiés par une compilation de méthodes spectroscopiques et chimiques. Les substances relevantes pour la nutrition telles vitamines, acides gras, minéraux et polyphénols majoritaires ont été quantifiés selon des méthodes standard.

La première espèce est connue sous le nom de *Phyteuma orbiculare* L. Les fleurs sucrées de cette plante sont réputées avoir été consommées par les bergers comme friandises, tandis que ses feuilles (rosettes) au goût de noisettes sont plutôt mangées sous forme de salade. Aucune étude phytochimique ni biologique n'était disponible ni pour l'espèce, ni pour le genre.

23 substances ont pu être identifiées des parties aériennes sèches incluant différents polyphénols, des acides gras et des triterpènes. Les investigations phytochimiques ont aussi débouché sur la découvertes de deux nouvelles saponines appelées phyteumosides A et B dont les sapogénines possèdent un squelette totalement nouveau et dont la chaîne glycosidique était également nouvelle. L'aglycone du phyteumoside A peut être considéré comme une molécule d'onocéroide incomplètement cyclisée tandis que l'aglycone du phyteumoside B possède un nouveau squelette 17-polypodène. L'identification de ces substances a pu être réalisée grâce à une compilation de données provenant de l'analyse MS/MS, HPLC-MS TOF, d'hydrolyses chimique et enzymatique, de RMN et de rayons X. En plus de ces deux triterpènes, un nouveau dimère dérivé du phénylpropane glycosylé a pu être isolé. Une comparaison des profils HPLC-MS DAD de la plante sèche et de la plante fraîche sont similaires.

D'un point de vue nutritionnel, *Phyteuma orbiculare* contient un taux important d'acide ascorbique, de β-carotène, de polyphénols, d'acides gras polyinsaturés, de potassium, magnésium et calcium. Cette plante, qui possède des propriétés nutritives intéressantes ainsi que des prédispositions favorables à une mise en culture, constitue une candidate intéressante pour un futur développement agronomique.

Il était alors intéressant de comparer son profil phytochimique avec des espèces du même genre présentant une biomasse plus importante. Une analyse HPLC-MS DAD a révélé un profil semblable pour *P. spicatum, P. ovatum* et *P. nigrum* mais différait pour *P. hemisphaericum*.

La deuxième espèce investiguée était un chardon appelé *Cirsium spinosissimum*. Le réceptacle est mangé au début de l'été. Les feuilles le recouvrant et les pappi sont enlevés avant consommation. Le goût du réceptacle ressemble à celui de l'artichaut et sa consistance est tendre.

20 substances ont été identifiées incluant différents polyphénols, un monoterpène lactone, des acides gras et un dérivé de la spermine. Les polyphénols majoritaires sont la linarine et la pectolinarine qui se retrouvent également dans d'autres espèces de cirses.

Cette plante contient des taux en vitamines standards, peu d'acides gras polyinsaturés, mais un bon taux de potassium. Cette plante ne se prête pas très bien pour une mise en culture future vue son caractère épineux, la difficulté de préparation et sa valeur nutritive dans la moyenne.

D'autres plantes alpines comestibles sélectionnées lors de cette thèse peuvent aussi se révéler intéressantes d'un point de vue autant scientifique qu'agronomique et faire l'objet de recherches futures afin de diversifier notre alimentation ainsi que l'agriculture de montagne.

1. Introduction

1.1 Nutrition

1.1.1 Health problems in Switzerland

Heart, metabolic, cancer, and respiratory diseases account for 60% of all deaths worldwide.[1] The World Health Organization (WHO) blew the whistle and started promoting interventions to reduce the main modifiable risk factors responsible for non-communicable diseases: unhealthy diets, physical inactivity, excessive consumption of alcohol, and tobacco.[2] These harmful behaviors are known to be associated with biological risk factors including high blood pressure, cholesterol, blood glucose, and excessive weight.

Even though a country like Switzerland seems to have a lower prevalence of obesity in comparison to other countries (e.g. United States, Greece, Slovenia), statistics from 2007 confirmed that 30% of the Swiss population suffer from being overweight (BMI > 25) and 8% from obesity (BMI > 30).[3, 4] Children are not immune to the epidemic, since 15-20% of them are overweight, and 4% obese.[5]

Increased relative weight coincides with disruptions in lipid and glucose metabolism, which translate into higher hypercholesterolemia and diabetes prevalence rates.[6-8] In Switzerland, prevalence of hypercholesterolemia and diabetes treatments doubled from 1993 to 2003.[9]

The rapid increase in the number of overweight and obese Swiss inhabitants and the resulting comorbidities are major health and political challenges.[3] At the 65th World Health Assembly held in Geneva in May 2012, health ministers pledged a 25% cut in premature deaths from the four most prevalent diseases mentioned above.[10]

1.1.2 Fruit and vegetable consumption to prevent non-communicable diseases

The WHO has suggested that a number of these killer diseases can be reduced by changes on lifestyle, by controlling tobacco use, by promoting a healthy diet and physical activity, and by reducing the harmful use of alcohol.[2] As far as the healthy diet is concerned, WHO experts encourage increased consumption of fruit and vegetables daily. Fruits and vegetables are an important element of a healthy diet and, if consumed daily in sufficient amounts, could help to prevent major diseases such as cardiovascular diseases and even certain cancer types.[11] Epidemiological studies corroborate all these assertions. Several classes of molecules synthesized in plants, particularly secondary metabolites, have been demonstrated to possess health beneficial properties against cancer or metabolic diseases.

Benefits of plants against cancer

Increased individual consumption of fruits and vegetables, to a *minimum* of 400 g per day, could prevent at least 20% of all cancer types such as those associated with the mouth, pharynx, esophagus, stomach, colon, and rectum.[12] The ability of dietary substances to inhibit tumor formation both *in vitro* and *in vivo* is largely documented. Many of these compounds possess anti-oxidant, anti-inflammatory, anti-proliferative, and pro-apoptotic effects on a variety of cancers.[13, 14] They may also act as angiogenesis inhibitors, block metastasis invasion, allow detoxification, or prevent absorption of carcinogenic products. Epidemiological evidence has correlated cancer risk reduction to the consumption of some Brassicaceae plants, garlic, onion, green tea, coffee, *Citrus* fruits, tomatoes, berries, ginger, and ginseng.[12] Main compound classes showing these pharmacological effects include carotenoids (lycopene), thiocyanates (sulforaphane issued from glucoraphanin, indol-3-carbinol resulting from the degradation of indol-3-methyl isothiocyanate), sulfur containing compounds (di- or tri-sulfides occuring from alliin), polyphenols (curcumin, epigallocatechin-3-gallate, resveratrol, genistein), and proteins (Bowman-Birk inhibitor).[15-18] No obvious toxic effects have been reported for most food substances. Thus, edible plants can be consumed in diets to either prevent primary tumor formation, or tumor recurrence.

Benefits of plants against cardiovascular diseases

Diets rich in fruit and vegetables showed a strong protective effect against stroke and reduced most metabolic risks.[19-21] A number of molecular mechanisms have been proposed. Antioxidants such as polyphenols, vitamins, carotenes, selenium, or zinc may have protective effects against the oxidation of low density lipoproteins (LDL) in atherosclerosis.[19, 22] Potassium and calcium, two main minerals that occur in fruit and vegetables, possess an important role in the regulation of blood pressure.[20, 23] In addition, phytochemicals have been shown to play a role in the reduction of platelet aggregation (resveratrol, quercetin) or modulation of cholesterol biosynthesis by inhibition of the HMG-CoA reductase (tocotrienol, alliin, dietary fibers, and carotenoids).[24, 25] Phytosterols can inhibit the absorption of cholesterol, while saponins and dietary fibers prevent the reabsorption of bile acids.[26, 27] Recent data suggested that phytochemicals with anti-inflammatory properties might be beneficial for prevention of cardiovascular diseases, since inflammation promotes the initiation and progression of atherosclerosis.[22]

In addition, the benefits of a diet rich in fruits and vegetables on cardiovascular diseases can be amplified by association with other healthy behaviors, such as lower intake of sodium, calories, and saturated fatty acids. In summary, diets rich in fruits and vegetables, as proposed in the Mediterranean diet or in the DASH diet, improve metabolic risk-factor profiles.

1.1.3 Promotion of consumption of fruits and vegetables

During the 6th congress of nutrition held in Switzerland in 2012, scientists reported that Swiss inhabitants consume around three portions of fruits and vegetables, which represents an inadequate amount according to international guidelines (five portions) .[28] A combination of policies and actions in order to be able to increase their intake in the population had to be planned.[29] Switzerland entered into national nutritional plans proposed by the WHO for example “Action Plan for the Global Strategy for the Prevention and Control of Non-Communicable Diseases 2008-2013”, “European Charter on Counteracting Obesity”, or “Action Plan for Food and Nutrition Policy 2007-2012”. According to the WHO recommendations and to “White Paper on a Strategy for Europe on Nutrition, Overweight and Obesity Related Health Issues”, the Swiss government created the “National Program for Nutrition and Physical Activity”.[30]

Diets providing a high variety of vegetables and a low amount of sweets, snacks, condiments, and carbohydrates may promote a long-term reduction in body fat.[31] Consequently, United States Department of Agriculture (USDA) promotes "to try new choices of vegetables and fruits".[32] Besides the variety, choice of food by consumers also depends on other issues such as sensory appeal. In the last few decades, repeated genetic selections in agriculture have in certain cases led to a loss of flavor of fruits or vegetables (*e.g.* tomatoes).[33] As a result of this process, consumers have often lost interest in eating these fruits and vegetables, which do not present the expected natural flavor traits anymore.

This is the reason why some forgotten edible plants with interesting gustatory properties and a phytochemical composition which might have beneficial effects on health, can be a source of inspiration to diversify our nutrition. Rocket salad (*Eruca sativa*) and false flax (*Camelina sativa*) are two cases for the successful development of locally used crops into mainstream diet.[34, 35] Consumption of wild edible plants is not any more a matter of survival, but nowadays rather considered as health food. In this context, species can be only temporary in vogue. For example, *Centaurea cyanus*, which was largely used in Poland as principal ingredient of fermented lemonade in the mid-20th century, fell in oblivion in this country.[36] In contrast, in Sweden, the use of *Sambucus nigra* and *Filipendula ulmaria* since 1970 for making "cordial" is still a widespread practice.[36]

Switzerland possesses a large panel of forgotten edible plants, especially in the Alps, where inhabitants have abundantly consumed wild plants as medicines or food. Ethnobotanical investigations in Swiss alpine regions on traditional plant knowledge should help to rediscover new sorts of fruits or vegetables.

1.2 Ethnobotanical considerations

1.2.1 Previous ethnobotanical studies in European countries

Wild botanicals used by indigenous communities in some European countries have been the object of ethnobotanical surveys.

In a recent issue of *Acta Societatis Botanicorum Poloniae* dedicated to ethnobotany of wild food plants, several reviews covered the use of edible plants in various countries, in particular in Eastern and Northern Europe. One of the contributions emphasized changes in the use of wild food plants which occurred during the last century mainly in Poland, Estonia, Sweden, Italy and Spain.[36] Its authors concluded that wild food plants are nowadays reserved for *haute cuisine* and as delicacies.[37]

In rural areas of Southern Europe the use of numerous species of wild leafy vegetables and spices has survived.[38] Rivera *et al.* reported fungi and vascular plants used as food and medicine by the Cimbrian ethnic minority which had spread along the Southern and Eastern borders of the Dolomites (Italy), and analyzed the results in the context of the alpine cultures.[39] Traditional knowledge about medicinal plants and food plants which come from the Northern part of Italy, in particular Lombardy was compiled by Vitalini and coworkers.[40] They showed that around 30 wild plants were used for food purpose, mainly as flavorings or ingredients of soups, omelettes, salads, and desserts. Schunko *et al.* investigated the Austrian organic food culture, while Christanell *et al*. performed a survey on food consumed by the Tyrolian population (Austria).[41-43]

Only a few studies on ethnobotanical uses have focused on Valais.[44-49] These studies aimed to list the plant names in local dialect or to investigate their medicinal traditional local uses. Different mainstream books written by elderly local people are available in French but have a limited area of diffusion.[50-53] These books mostly deal with medicinal and edible plants and provide personal recipes and practical advice. Thus far, there is little ethnographic literature on the use of wild plants as food sources in the canton of Valais. A master thesis documented plants used only in the Val d'Anniviers as medicines or as food, as work tools or as toys.[54] Its author interviewed 30 inhabitants of Val d'Anniviers and could identify 56 plants traditionally consumed as food in the valley.

1.2.2 Methodology

Selection of the informants

Further investigations could consequently be undertaken in other parts of the canton as for example in Val d'Entremont, Val d'Illiez, and Val d'Hérens. A fundamental aspect of any ethnobotanical study lies in the selection of suitable informants, who can be suggested by community members (relatives, friends, community leaders). This approach facilitates receptiveness during data collection, as informants feel that their participation has been locally endorsed and only targets people recognized as knowledgeable by the community.[55, 56]

The researcher must be able to talk about the concepts under discussion (e.g. food plants) and should consequently show linguistic fluency and understanding of local dialect. He should be endowed with sociable personality and social standing in the region.[55, 57] In the present work, the author was native to the canton of Valais and could quickly create a network essential to identify some local knowledgeable locale inhabitants.

Considering that the peoples' dependence on folk medicines and use of wild plants had declined considerably since the 1940s, members of the older generation (defined as age 70 and above) were specifically targeted.

Interview technique

As soon as the informants have accepted to be interviewed, a number of key anthropological and botanical methodologies must be set up to collect a maximum of pertinent information. The researcher generally combines quantitative and qualitative methods to ensure the proper collection of data. Interview techniques play a key role to obtain information from knowledgeable locale inhabitants. The technique has to be chosen according to the purposes of the study.

In the context of our study aimed at shortlisting plants used in the alpine tradition, semi-structured interviews, which are based around a checklist of topics or questions (e.g. mode of use of food plants), seemed to be best suited. This methodology allows a broad range and depth of information to be revealed, which is difficult to elicit using more formal methods (e.g. structured interviews). Semi-structural interviews, additionally, create a somewhat

informal partnership between informants and researcher, which facilitated sharing information.

1.2.3 Ancient bibliographic documentation

The consumption of many wild vegetables or snacks in the canton of Valais was often linked to herding. When following cattle or goats, children herders could observe the nature and tried to taste traditional snacks. As most of these activities are not common any more, people have abandoned the behavior they had during pasture times. Research in ancient treaties written during the time, where agriculture played an important role in families living in the canton of Valais, can bring out forgotten wild edible plants or corroborate traditional uses of certain species still consumed nowadays.

For this purpose, two local libraries (Centre Régional d'Etude des Populations Alpines, CREPA in Sembrancher, and Hospice du Grand-Saint-Bernard, Bourg-Saint-Pierre) were searched for relevant ethnobotanical literature. The private library of Grand-Saint-Bernard has kept a large panel of ancient books written in previous centuries (mostly from XVIIth to XIXth s.). Some books that focus on plant tradition of Valais were selected for intensive reading. These books were written by notables including priests, professors, and medical doctors.

1.3 Alpine plants

1.3.1 General considerations

Etymology of the word *alpine* comes from the Latin "*albus*" which means "white" or "snow-covered". Some linguistic experts propose to also consider the pre-Roman origin "*alp*" or "*alb*", whichstands for "mountain" in general.[58] An alpine plant is defined as a species growing above the climatic treeline (alpine life zone). Alpine flora of the world is estimated to several thousand species of higher plants.[58] European alpine mountains flora includes around 600 to 650 genuine alpine species.[58] Alpine plants are mostly flowering plants (Spermatophyta) and bryophytes, whereas ferns are represented by a few species. They are regrouped in ten principal growth forms including cushion plants, prostrate woody shrubs, graminoids, and herbaceous perennials.[58]

The systematic composition of the alpine flora is obviously different from that of the lowland regions. Brassicaceae and Cyperaceae are over-represented, whereas Lamiaceae and Apiaceae are under-represented.[57] The genera *Androsace*, *Artemisia*, *Astragalus*, *Campanula*, *Cerastium*, *Draba*, *Gentiana*, *Pedicularis*, *Phyteuma*, *Potentilla*, *Primula*, *Ranunculus*, *Salix*, *Saxifraga*, and *Viola* constitute about 34% of Valais flora or 30% in Swiss Alps.[59] However, as a result of water availability, rough terrain, relative isolation, and microhabitats, mountains create altitudinal segregated life zones at short distances and physical barriers to genetic variation, which are conditions favoring the development of high species diversity.[60]

1.3.2 Altitudinal conditions

The roughness of mountain topography, sunny meadows, ravines, river banks, and other features create niches offering diverse resources and climates to the alpine vegetation. Plants must cope with life-threatening abiotic conditions changing not only on yearly cycles but also on a daily basis. Hard conditions include factors like temperature, wind velocity, atmospheric gas composition, water availability, nutrient deposition, soil weathering, and solar irradiance.[60] Reduced CO_2 partial pressure limits the carbon supply for photosynthesis, whereas soil nutrients occur in low concentrations at high elevation, limiting plant growth; water in solid phase can cause water stress, while solar irradiance in the UV range may reach

energy levels sufficient for breaking the bond of organic molecules and biopolymers such as DNA; early freezing events may cause loss of the immature seed crop of late flowering species or injuries in the leaves .[58, 60]

1.3.3 Strategies of adaptation

Tolerance and avoidance are the two major diverging strategies used by plants under stressful conditions. Avoidance entails traits that enable plants to resist adverse conditions by preventing the deleterious effects of these conditions, whereas tolerance is characterized by the organism confronting the molecular damage and repairing it.[60, 61] Characteristics of these strategies in alpine plants are explained in the further paragraphs.

Tolerance

Tolerance strategies consist of traits that enable plants to endure adverse conditions and include chemical synthesis of antioxidants, modulation of enzymatic activity or of membrane hardiness.

Enzymatic activity. Altitudinal lower temperatures and UV-B radiation may influence the activities or stability of certain enzymes. For example, the polymerase II found at high altitudes, which contributes to the reparation of DNA damage, shows a greater stability than its relatives belonging to plants growing in a lower elevation, while enzymes involved in free radical scavenging capacity or antioxidant mechanisms (e.g. dismutase, glutathione reductase, and ascorbate peroxidase) exhibit increased activities in the presence of UV-B. Interestingly, either low temperature or light can induce the expression of enzymes involved in the synthesis of some phenolic compounds like flavonoids.[60, 62, 63]

Antioxidants. The leaf epidermis effectively protects the mesophyll from UV-B exposure.[58] Production of efficient antioxidant molecules were suggested to be correlated with oxidative stress produced by UV-B exposure. Among the compounds responsible for UV-B absorbance, flavonoids and hydrocinnamic acid conjugates are the most important metabolites found in epidermal and upper parenchymal cells which obstruct UV-B penetration. Glutathione synthesized at relatively high levels is also known to act in concert with ascorbate and to protect labile macromolecules by scavenging free radicals and hydrogen peroxide.[60, 64] Anthocyanidins contained in reddish leaves during the cold season, carotenes, stilbenes,

lignans, tannins, and waxes are reported in literature as further protective agents.[58] Various isoquinolizidine and triptophane-derived alkaloids, as well as metabolites issued from the phenylalanine deaminase pathway, which absorb radiation in a range of 210-350 nm, may also be useful against UV-B.[60]

Hardiness of membranes. Hardiness of membranes varies according to the development phase. During the dormancy state in winter, the hardiness is maximal in persistent tissue, whereas a minimum resistance is found during the summer time. This seasonal trend allows the plant to acquire heat tolerance.

Avoidance

Avoidance strategies involve different various genetically controlled traits including plant size, large roots' systems used as storage site of carbohydrates, production of fatty acids, leaf histology, and supercooling phenomenon.

Small size. Species growing in mountainous regions are smaller and more dwarfed than their congeners of the plains (Figure 1).[65] The short vegetation season lasting one to three months force alpine plants to complete vegetative and reproductive cycles in a short time lapse. The plant must find the optimal carbon balance between growing and reproducing.[66] Small sizes may be of crucial importance against frost threat. In regions with winter snow cover like in the Swiss Alps, tall plants are more at risk than smaller plants with respect to the damage causing by freezing, since smaller plants can profit during the night of re-irradiations of heat accumulated in the ground along the day.[58] This strategy of prostrate growth to recover heat along the night can however cause overheating during the day. Time without sun or bad weather conditions, which often occur in alpine areas, may also severely constrain leaf growth, and consequently the investment of carbon assimilates.[58] Alpine plants' leaves are closer together, more hairy, relatively thicker and smaller, and colored with a much deeper green than those of lowland plants.[65]

Supercooling. Some taller alpine plants can resist frost damage by supercooling. This strategy exists to substantially cool leaf and stem tissues below freezing point without freezing and consequently to avoid nucleation.[58] This mechanism is more effective but more risky than osmotic adjustment induced by sugar accumulation. When temperatures below supercooling capacity occur, the process becomes a fatal strategy because of abrupt frost

occurring in the organs. Large extracellular mucilage content found normally in leaves helps to avoid the possible damaging effects caused by supercooling.[58, 67, 68]

Meristems below ground. The stem of alpine plants is generally shorter and more buried in the earth (Figure 1).[65] Roots of most alpine dicots possess a deep primary root system with shoots proliferating near the soil surface.[69] Interestingly, underground parts have a greater dry weight than shoots (2-6 times greater).[69] The large meristem network represents a survival strategy for the plant. In hard conditions such as late frost in spring, avoidance and tolerance mechanisms may be insufficient. Frost damage will occur and the plant will lose aerial organs. In such situations, survival of the organism depends on its quick repair and replacement abilities. The preformation of future leaf cohorts and sufficient storage reserves in roots are crucial for a second flush.

Large content of fatty acids and carbohydrates. The large root system of alpine plants contains substantial reserves of carbohydrates and fatty acids.[58, 70] The reserve of carbohydrates is normally utilized in the rapid growth of the shoot in early summer. At this time, growth is so rapid that respiration exceeds photosynthesis and a large amount of nutrients is needed, which depletes the reserve of carbohydrates.[71] Accumulation of sugars has an important role in osmotic adjustment and results on a freezing point depression. Sugars for example act also as source of energy and "cryoprotectors" on biomembranes.[58] Special lipids alter the flexibility and the water permeability of the tissues by modifying cell membranes.

Waxy cuticles or wooly leaves. Alpine plants possess different idiosyncratic characteristics to prevent from the penetration of UV light into sensitive areas of the plants. Waxy cuticles in glabrous leaves and conifer needles, or pubescent layers covering the adaxial epidermis, attenuate incident UV-B photons to 5% transmittance or less.[60, 72-74] Hairs are efficient phototonic structures, which influence the reflectance and the transmittance of UV light reaching the mesophyll.[60] The edelweiss (*Leontopodium alpinum* Cass.) for example possesses wooly protection on the bracts, which show significant reflectance capacity of 60% of incident light above 400 nm.[60]

Alpine plants have developed other avoidance strategies. Selection of topographically safe sites, hiding under snow or litter, and withdrawing sensitive tissue during the cold season from exposed positions are some of these examples.[58]

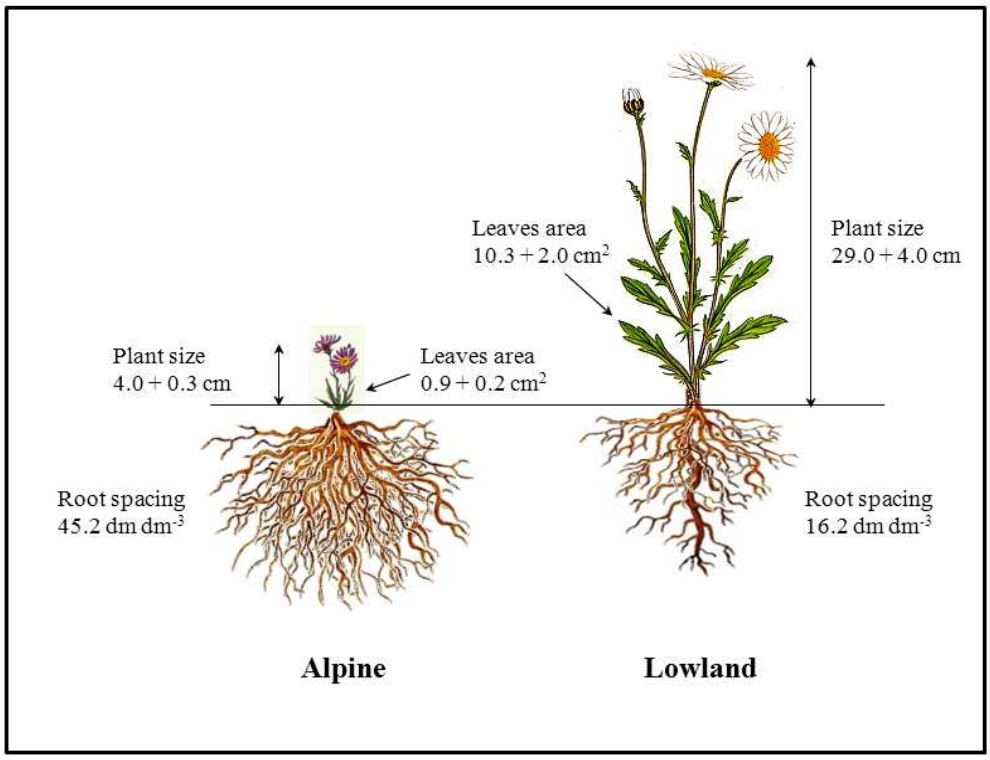

Figure 1. Comparison between alpine and lowland plants. Alpine plants and their leaves are significantly smaller than their relatives of lower altitudes. Alpine fine root biomass and their root length are greater.[75] Roots are more spread out.

1.3.4 Breeding and cultivation of alpine plants

Consumption of herbal medicines is widespread and still increasing. The use of herbal medicines is especially growing in developed countries: herbal and traditional products increased in value by 2% in 2011 in Switzerland;[76] 25% and 20% of the population were advised to take herbal medicines regularly in UK and France respectively.[77, 78] This trend for natural products requires agriculture to provide sufficient amounts of plants. Agricultural cultivation actually includes several obvious advantages in comparison with harvesting from the wild. The botanical identification is more reliable, whereas the genetic, phenotypic, and phytochemical variability are reduced. The raw material is supplied in sufficient amounts and agricultural works are conducted according to the Good Agricultural Practices (GAP) guidelines, which guarantee appropriate conservation, traceability, and stability of the plants.[79] Scientist efforts are towards improving conditions for seed germination, plant growing (planting, fertilization, and irrigation), harvesting, drying, and conservation.[79] Controlled growth systems allow modification of the concentration of substances relevant for

nutrition or medicine (e.g. artemisinine in *Artemisia annua*, thymol in *Thymus vulgaris*) and to reduce levels of toxic compounds (thujone in *Artemisia umbelliformis*).[80] It creates higher resistance against biotic and abiotic factors (infestation of *Hypericum perforatum* by *Colletotrichum cf. gloesporioides* or *Artemisia* sp. by *Puccinia absinthii*) and aims to obtain uniformity of the cultures (*Salvia officinalis*).[80] These processes allow a good predictability of extracts.

In Switzerland for example, around 250 ha are delegated to medicinal and aromatic plant breeding, which provide around 350 tons of aromatic and medicinal herbs.[81] While this surface represents *circa* 0.03‰ of the Swiss area (around 20 times less than Germany or Austria (both 0.7‰)), Switzerland favors organic farming (80% of its culture areas). The cantons of Valais (Valplantes) and Bern (Emmental, Waldhof) are the main Swiss regions that manufacture plants. Minor production takes place in Graubunden (Coperme), Lucern (Entlebuch), Tessin, Jura, Basel (Laufen), and Vaud (Bassins).[81]

Plant breeding techniques/technologies

Two main strategies for plant breeding are used: first creating a maximum number of variations and then, among the available variability, choosing individuals that meet the breeding objectives.[82]

Creation of variability. A breeding program starts with the choice of an initial population obtained from existing cultivars and endowed with desirable traits (frost resistance, heat tolerance, *etc.*). To optimize hybridization results, parents must have a maximum number of positive traits and a minimum of negative traits with no negative trait in common. The final aim of crop improvement is consequently to involve the transfer of genes from the desired genetic background source to another one, with the hope that individuals in the next generation will possess the best features of each parent.[83] The F1 generation is used as final product for seed commercialization due to its high homogeneity.

Artificial pollination, hybridization, tissue culture, chromosome doubling, bridge cross, or DNA technology are only a few examples of techniques that have been used in the quest for desired variation. Many cultivars of medicinal and aromatic plants (MAPs) proposed on the Swiss market were obtained by hybridization of genetically different plants.[84-86] Certain basic factors must be considered in the preparation for hybridization. Parents should belong to the same or closely related species and supply the critical genes needed to accomplish the

breeding objective. One parent is generally emasculated by removing anthers and is then designated as female.[83, 85] Extensive genetic variability within a crop species generally occurs in clusters within small geographic regions separated by geographic features such as mountains, rivers, and deserts. In Switzerland, alpine regions, which possess different microclimates, therefore may provide many parents which are genetically different.

Assembly of two genomes into a newly created individual may create heterosis, where genes of the hybrid can complement each other and enhance the vigor of the hybrid.[83] For example, during experiments in Valais, hybridization of two sorts of thyme gave a hybrid as rich in essential oil as its Mediterranean parent and as frost resistant as its German parent.[84] Another key point of hybridization is a transgressive segregation, where hybrids have features representing an average of the parental features, or a bias toward the features of one parent.[83] Rey *et al.* found a thujone free cultivar of *Artemisia umbelliformis* in the region of Mattmark (Valais), whereas the sweeter tasting population growing in Simplon (Valais), a neighboring region, contained an important content of thujone.[80] Hybridization of both of them gave a cultivar with moderate legal content in monoterpene, which could maintain typical taste of the drink.

Selection. Various strategies have been developed for selection in breeding programs. Breeding schemes are distinguished by the nature and source of the population used to initiate the breeding program, as well as by the nature of the product. Other techniques can use markers. Markers are defined as phenotypes that are linked to genotypes. They facilitate the selection and are cost-effective. Finally, gene mapping allows a selection of hybrids which possess located and identified genes conditioning a trait of character (e.g. gene encoding for drought stress or cold stress).[83]

Challenges in alpine plant breeding

One of the main obstacles to bring a plant into cultivation is the long duration of the breeding process, which typically takes 5 to 15 years before obtaining plants on the market.[80] In the future, methods to accelerate the breeding process must be taken into account to respond more quickly to the requirements of stakeholders.[79]

Each alpine plant breeding program is different with its own challenges and opportunities. It is quite difficult to foresee how a plant will grow in predetermined conditions. An individual plant collected from the wild can be sensitive to a specific disease and develops

this sensitivity more extensively when cultivated. For example, *Hypericum perforatum* was easily infested by an anthracnose caused by *Colletotrichum cf. gloesporioides* or cultivars of *A. umbelliformis* containing large amounts of thujone were often attacked by the fungi *Puccinia absinthii*.[87, 88]

Previous criteria of selection can be applied to successful breeding. Even though small plants can double in size when put into cultivation, bigger and non-creeping plants are in general preferred for further successful cultivation. In addition, plants flowering several times a year possess obviously considerable advantages.

Opportunities for alpine plant breeding

Wild relatives of cultivated plants and especially alpine plants provide a reservoir of potentially relevant genes for crop improvements. High alpine biodiversity should allow the discovery of parents endowed with genotypes possessing key genes coding for important traits.[80] In the plant, the commonly targeted secondary metabolites frequently serve as adaptations to fluctuating temperatures and light conditions (e.g. antioxidant), stress (proline), infection (flavonoids), or herbivores (alkaloids).[77] Their accumulation depends on environmental factors such as water availability, exposure to soil microorganisms and variations in soil pH and nutrients.

The growth of leaves at high altitudes seems to be controlled in a way that leads to comparatively rich metabolite contents.[89] This assessment was corroborated by studies on phytochemical investigations on alpine pastures. Dihydroxycinnamic derivatives were detected in large amounts in pasture plants by colorimetric methods and thin layer chromatography.[90, 91] Several studies showed that the proportion of soluble phenolics increased with altitude or that representative mountain plants (e.g. *Tragopogon pratensis*, *Knautia arvensis*) contained large amount of phenolic compounds. Analysis of animal products demonstrated a relatively high proportion in polyunsaturated fatty acids, carotenoids, or terpenes.[92] Körner attested that “inherent development growth constraints inhibit nutrient dilution in the plant body and thus defy the application of plant-nutrient *versus* soil-nutrient”.[89]

1.4 Valais

1.4.1 MAPs cultivation in Valais

The canton of Valais was mentioned above to be the most MAPs producer in Switzerland since the canton accounts for almost 70% of the total Swiss production.[93] The canton of Valais and mountain farming must deal with small plots, significant slopes, difficult mechanization, and low yields. However, thanks to its microclimate, this region is particularly adapted to crop cultures. Muddy and sandy soils of the Rhone Valley are very fertile, whereas low rainfall (600 mm/year), strong sunshine (2'000 hours/year), and the good field irrigation favor an optimal development of fruits and vegetables species.[94]

The cultivation of MAPs in the canton of Valais took its origin from political and economic considerations. Until 1982, the Swiss needs of MAPs depended almost exclusively from abroad (India, Europe, and Algeria). Despite its higher production cost, Switzerland can compete in the market against its neighbors whose fees are much lower. Quality of its cultivars, bucolic image conveyed by the Alps and the stringent standards required by organic farming are the main strengths of the Swiss and Valais production.[81]

While Switzerland spends 90% of its cultivated area to provide turnip, cereals, sugar beet, and potatoes, Valais favors arboriculture (6%, 1300 ha) and viticulture (13%, 4976 ha).[95, 96] MAPs' cultivation remains niche crops in Valais. The hill on the right bank of the Rhone and the Val d'Entremont are the most important MAPs providers in Valais. These two regions possess different climates: dry or temperate, respectively. The German part of Valais features scattered sites, but is famous for its saffron harvesting in Mund. Valplantes is the main cooperation in Valais, which handles the production and marketing of MAPs. This organization delivers a large amount of herbs for the food industry and especially for the candy production (70%), teas (12%), spices (2%), cosmetics (1%), and for other drinks (15%).

1.4.2 Scientific network in Valais

The canton of Valais is a crossroad of knowledge regarding plants. Political, economic, and scientific synergies allow the canton of Valais to develop an interesting and promising

concept around medicinal plants. A site in Conthey includes on one hand a federal agricultural station for plant breeding assays and on the other hand PhytoArk an “incubator” to link academia and industry and to allow the installation of companies involved in processing of herbal products.

1.4.3 Plant use knowledge

The Valais population has still secular knowledge in medicinal and food use of plants. Unfortunately, this traditional knowledge is slowly falling into oblivion as a consequence of the deep societal and economic changes which have taken place over the last few decades. Until the middle of the 20th c., the absence of medical doctors, poverty, and the difficulty to travel made the inhabitants of side valleys reliant on plants as medicines or as food. The majority of the population lived of agriculture (57.8% in 1914) and had a strong interaction with nature.[97] Agricultural activities of young shepherds are often in line with recreational consumptions of wild food plants (Fig. 2).[37]

Figure 2. A) Agricultural activities were linked to consumption of food plants in villages. B) When following cattle, sheeps, or goats, children herders had a lot of time to observe nature, as they moved through the landscape. They also could taste different snacks.

Nowadays, the majority of the 50-60 years old population can recall some words and basic customs of their heritage, but do not incorporate them into their daily life. This trend is unabated in the younger generations (less than 50 years old), which have almost completely abandoned the traditional agro-mountainous way of life as a principal source of income to work mainly in the secondary or tertiary sectors.

1.4.4 MAPs cultivations, an interest for large public

The cultivation of alpine plants is not beneficial only to food, pharmaceutical or cosmetics industry, but may also affect other fields such as tourism. Valais, where tourism accounts for about 25% of GDP and employs 30% of people, must renew itself and find innovative ways to attract tourists.[98] The promotion of alpine plants may be an asset to the canton. Pharmacobotanical trails as well as alpine botanical gardens have already been developed by specialists and various guided tours as well as presentations done by experts are offered each year to local population and visitors. At a time when the soft tourism is gaining importance, Valais is interested in exploiting its flora and cultures of alpine plants to meet the expectations of a certain category of tourists.

Cultivation of MAPs offers a broad range of opportunities. This activity has grown considerably during the last decades to meet the demands of the industry, offering an alternative to mountain farmers.

2. Aim of the work

The first aim of this work was to identify food plants consumed traditionally in Valais. A robust method should be developed for collecting a maximum of information. This includes identification and contacting of knowledgeable inhabitants, as well as design of a suitable interview strategy and analysis of the collected information. In order to retrieve further plants which may have already fallen in oblivion, several books published in the XIXth or early XXth century should be also consulted.

In a further step, a selection of plants which appear to be promising candidates for cultivation should be submitted to a comprehensive phytochemical investigation. Plants should be selected based on chemotaxonomic considerations with regard to the expected absence of compounds with known or suspected toxicity, their ability to be domesticated, their economical potential, a convenient mode of utilization and pleasant gustatory properties.

Selected plants will be submitted to a comprehensive metabolite profiling using a dereplication platform combining HPLC-PDA-MS, and offline NMR analyses. In a first step, extracts of different polarities obtained from the dried plant material will be investigated to allow the identification of as many as possible secondary metabolites. Then, the phytochemical profile of the fresh edible organs will be determined with the aid of HPLC-PDA-ESIMS analyses.

Particular attention will be paid to substances relevant to nutrition, such as ascorbic acid, β-carotene, polyunsaturated fatty acids and minerals. These substances will be quantified in the fresh edible parts. The total amount of polyphenols and the content in the main flavonoids and other polyphenols will be also determined, since these metabolites are known to possess marked antioxidative properties which are of relevance in the context of the prevention of major lifestyle diseases.

Finally, a preliminary assessment of the potential of these plants for further development should be made based on the data obtained in this study. This will also include, if appropriate, the determination of the phytochemical profiles of systematically closely related species which are characterized by a larger biomass and may consequently be more promising from an economic perspective.

3. Results and discussion

3.1. Ethnobotanical survey on wild alpine food plants in Lower and Central Valais (Switzerland)

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Swiss Alps have an ancestral tradition with regard to the use of wild plants as medicines and food. However, this traditional knowledge is falling into oblivion, and is nowadays confined to village areas. This publication aimed to capture this patrimony in a written form to ensure its preservation.

In a preliminary work, 594 edible alpine plants have been shortlisted from the reading of main stream literature and ancient treaties. Around 10% of them were found to be potentially toxic in the short or long term, 22% were well established food, and 2% would be extremely difficult to be taken into cultivation.

Semi-directive interviews transcribed by taking notes or tape-recording were conducted in four different side valleys of the canton. 38 informants, whose ages range from 28 to 102 years, reported 98 food plants. 707 responses corresponding to the food use of plants were classified according to eight different modes of consumption. Teas were the most cited way of use. *Taraxacum officinale* is the culturally most important plant in canton of Valais according to the cultural importance index. *Sambucus nigra*, *Urtica dioica*, *Picea abies*, and *Vaccinium myrtillus* are other examples of wild plants often used as food. Interestingly, parts such as receptacles of *Carlina acaulis*, *Cirsium spinosissimum* and buds of *Heracleum sphondyllium* belonged to more original snacks traditionally consumed in Valais.

Books and reports written in the XIXth c., which contain information on the ancient use of plants in Valais, enabled to identify 38 further species consumed in the past but not cited

anymore by the informants. They include interesting plants such as *Bunium bulbocastanum* eaten as snack during ploughing times. The numerous edible wild plants identified in the course of this study prove the rich tradition still present in lower and central Valais and might provide interesting opportunities for further diversification of mountain agriculture.

The study consists of a compilation on one hand of a master's thesis led at the Conservatory and Botanical Gardens of the City of Geneva (CJB) (Switzerland) by Romain Mayor and on the other hand of field work completed in the beginning of my PhD thesis. I did interviews in Val d'Entremont, Val d'Anniviers, and Val d'Hérens, whereas Romain Mayor dealt with Val d'Illiez. Reading of ancient treaties, compilation of the ethnobotanical data, preparation of the figures, and writing of the manuscript were also my contributions for this paper.

Christian Abbet

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Ethnobotanical survey on wild alpine food plants in Lower and Central Valais (Switzerland)

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ABSTRACT

Ethnopharmacological relevance: Swiss Alps have an ancestral tradition with regard to the use of wild plants as medicines and food. However, this knowledge is falling into oblivion, and is nowadays confined to village areas. Aim of the study was to identify wild edible plants used today and during the last two centuries by the alpine population of Valais (Switzerland).

Material and methods: Data were collected by means of semi-directed interviews made in four different lateral valleys of Valais (Val d'Anniviers, Val d'Entremont, Val d'Hérens, and Val d'Illiez). Wild food plants were classified according to their uses (salads, cooked vegetables, spices, raw snacks, teas, alcoholic drinks, sirups, and jams). Books and reports written in the XIXth century were consulted to identify uses of wild plants which have fallen in oblivion meanwhile.

Results: A total of 98 edible wild plants, distributed into 38 botanical families, were identified during the interviews. Several plants were highly cited (e.g. *Taraxacum officinale*, *Chenopodium bonus-henricus*). The most frequent usage was as tea (18%), followed by uses as cooked vegetables (16%), jams (16%), and raw snacks (16%). A strong association was observed between food and medicinal uses of plants. Wild food plants were of critical importance in times of food scarcity. Meanwhile, they have lost their relevance as vital components of the diet and are nowadays rather perceived and appreciated as delicacies.

Conclusions: This study provides for the first time comprehensive data on present day and historical uses of wild plants as food in Lower and Central Valais. Besides being of historical interest, this ethnobotanical information can be used to identify species which may provide interesting opportunities for diversification of mountain agriculture.

1. Introduction

The strong connection between nutrition and health is increasingly recognized (Etkin, 1996). Food plants are not solely considered from a nutritional perspective, but also as a factor of prevention of most prevalent lifestyle diseases, such as diabetes, cardiovascular diseases and cancer. According to modern nutritional studies the consumption of leafy vegetables brings numerous health benefits, and their everyday consumption in diet is highly recommended (Block, 1991). When compared to cultivated vegetables, wild vegetables may have some nutritional advantages, since their content in polyunsaturated fatty acids, and vitamins E and C is usually higher (Simopoulos, 2004).

Some traditional edible plants with favorable pharmacological or nutritional properties can offer new opportunities for the development of mountain agriculture if they can be taken into cultivation. Rocket salad (*Eruca sativa*) is an example of the successful development of a locally used food plant into an international and well appreciated salad (Padulosi et al., 1997).

Wild botanicals used by indigenous communities in some European countries have been the object of ethnobotanical surveys. In a recent issue of *Acta Societatis Botanicorum Poloniae* dedicated to ethnobotany of wild food plants, several reviews covered the use of edible plants in various countries, in particular in Eastern and Northern Europe (Luczaj, 2012). One of the contributions emphasized changes in the use of wild food plants which occurred during the last century mainly in Poland, Estonia, Sweden, Italy and Spain. (Luczaj et al., 2012). In rural areas of southern Europe the use of numerous species of wild leafy vegetables and spices has survived (Pieroni et al., 2005). Rivera et al. repertoried the fungi and vascular plants used as food and medicine by the Cimbrian ethnic minority which had spread along the southern and eastern borders of the Dolomites (Italy), and analyzed the results in the context of the alpine cultures (Rivera et al., 2006). Traditional knowledge about medicinal

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plants and food plants coming from the Northern part of Italy, in particular Lombardy was compiled by Vitalini and coworkers (Vitalini et al., 2013). Schunko et al. investigated the Austrian organic food culture, while Christanell et al. performed a survey on food consumed by the Tyrolian population (Austria) (Christanell et al., 2010; Schunko and Vogl, 2010). In his pioneering work on the uise of food plants in history, Adam Maurizio, a Swiss–Polish economic botanist and food scientist of the XIXth and early XXth century, reported nutritional habits of farmers in Switzerland (Maurizio and Gidon, 1932). So far, there is little ethnographic literature on the use of wild plants as food in Valais. A master thesis documented plants used in the Val d'Anniviers as medicines or as food, as work tools or as toys (Brüschweiler, 1999). Some studies on ethnobotanical uses have been focusing on Valais (Nicollier and Nicollier, 1984; Roguet, 1991; Roguet and Spichiger, 1994; Perrenoud et al., 1998; Mayor, 2002; Roguet, 2004). Different mainstream books written by elderly local people are available in French but have a limited area of diffusion (Cousin-Zermatten, 2005; Favez, 2007; Cousin-Zermatten, 2009, 2010). These books deal mostly with medicinal and edible plants and provide personal recipes and practical advice. However, no data are provided with regard to the history and frequency of use. The geographic areas where the plants are consumed are also not indicated.

The present study is a compilation of data collected in an ethnobotanical study conducted by the Conservatory and Botanical Gardens of the City of Geneva (CJB) (Switzerland), and three similar surveys carried out by the University of Basel (Switzerland). These investigations covered four different parts of Valais (Val d'Anniviers, Val d'Entremont, Val d'Hérens, and Val d'Illiez), and data collection was done with the aid of interviews. In a further step, books, and reports from the XIXth century on traditional uses of plants in Valais were consulted. They allowed us to identify additional plant species that had been consumed in the past, but were not anymore cited by the informants.

2. Material and methods

2.1. Study area

The surveys were carried out in Central and Lower Valais. As one of the 26 cantons of Switzerland, the Valais is located in the southwestern part of the country, along the Rhône valley, from the headwaters to the Lake of Geneva, and it separates the Pennine Alps from the Bernese Alps. These mountain chains protect the Rhône valley and its lateral valleys from the humid conditions often prevailing in adjacent areas. The Alpine grasslands are characterized by ecosystems such as steppes, high altitude areas, and damp environments with a great diversity of plant species. The national institution responsible for the mapping of the Swiss flora listed 1841 and 879 plants species growing in Valais below and above 1300 m above sea level, respectively (WSL, 2006).

The Valais has approx. 320,000 inhabitants including 20% of foreign nationals (Swiss Confederation, 2013). More than two-thirds of the total population of Valais is French-speaking, while in the eastern part of the canton (Upper Valais), a characteristic German dialect is spoken (Walliser German). In some lateral valleys of Central and Lower Valais, the population continues to speak local French dialects which differ from one valley to the other. These valleys regroup few villages which are sustained primarily by agriculture, tourism, and revenues from hydroelectric power plants. Viticulture is important in the plain of the Rhône river, whereas cultures of strawberries, and herbs for cosmetic (e.g. Edelweiss), medicinal and food industries (e.g. sage and thyme) are found in the lateral valleys. Inhabitants are also used to cultivate their own vegetables and fruits.

Up to the mid of the XXth century, the lack of professional healthcare, poverty, and the difficulty to travel made the inhabitants of side valleys reliant on plants as medicines or as food. The majority of the population lived of agriculture (57.8% in 1914 (Canton of Valais, 2013)), and thus had a good understanding of the natural resources of their surroundings. As a consequence of the deep societal and economic changes that have taken place over the last decades, this traditional knowledge tends to fall into oblivion. In fact, the majority of the 50–60 years old population can recall some words and basic customs of their heritage, but do not incorporate these into their daily life. The younger generations (less than 50 years old) have almost completely abandoned the traditional agro-mountainous way of life as primary source of income and work mainly in the secondary and tertiary sectors.

2.2. Informants and interviews

Investigations were conducted between 1999 and 2011 in four rural and mountainous areas of Valais, namely Val d'Entremont, Val d'Illiez, Val d'Hérens, and Val d'Anniviers. These regions were selected because of their cultural and botanical richness. In the process of recruiting informants, efforts were made to identify people regarded in their communities as particularly knowledgeable on traditional uses of plants as medicines or food.

All informants lived in hamlets with a population of less than 2000 inhabitants. Most informants were members of the Swiss middle class, and most of them had experience with agriculture. The majority were retired. Names of plants were cited in local dialect or in French. Identity of the plants was confirmed with the help of Flora Helvetica and Flore de la Suisse (Lauber and Wagner, 2001; Aeschimann et al., 2005). Interviews were managed with semi-directive interviews (Appendix A) and transcribed by taking notes. The study covered a time frame from 1910s to 1920s to the present. Considering that the peoples' dependence on folk medicines and use of wild plants had declined considerably since the 1940s, members of the older generation (defined as age 70 and above) were specifically targeted. More females than males were interviewed. Even though this bias was not intentional, it was in accord with the generally accepted observation that women traditionally had to take care of food and medicine (Table 1).

2.2.1. Val d'illiez

Ethnobotanical investigations were carried out by Mayor. He contacted key informants through parsons and school children. He also published an advertisement in a regional newspaper to inform about the aim of his study, and to encourage indigenous inhabitants to participate to the survey. Interviews were designed to gather information about past and current uses of plants. If an interview of 2 h was not sufficient to clarify all points, a second appointment was scheduled. Occasionally, a walk through fields or woods was conducted.

2.2.2. Val d'Entremont, Val d'Anniviers and Val d'Hérens

The surveys were undertaken by Abbet. The author is native from Val d'Entremont and knew people from this valley possessing information about past and current uses of wild food plants. For other valleys, informants were recommended by locale inhabitants of the valley. All informants were well reputed for being knowledgeable herbalists. Abbet used for his surveys a semi-structured interview approach (see Appendix A). A questionnaire was sent a week before the interview to selected inhabitants. A meeting was then scheduled to follow up the data contained in the questionnaire. In some cases plants were collected together with the informant, to confirm the correct way of use and to taste them.

Table 1
Characteristics of ethnobotanical surveys.

District	Entremont	Anniviers	Hérens	Illiez
Inhabitants[a]	14,410	2611	10,363	1763
Total informants (*n*)[b]	17	3	1[c]	17
Males (*n*)	8	0	0	5
Women (*n*)	9	3	1	12
Age range	28–102	50–89	84	50–95
Age median	70	71	84	73
Interviewer	Abbet	Abbet	Abbet	Mayor
Year of the survey	2009–2011	2009	2009	1999–2000

[a] Data from the Federal Annals (Swiss Confederation, 2012).
[b] Different numbers of informant were considered to be acceptable, as the survey did not aim at a comparison between valleys.
[c] Native person, reputed for having collected data about all food plants of the valley and considered as a reference person for plant-related issues.

The completed questionnaires and tape recordings are kept at the University of Geneva – CJB (Val d'Illiez), and at the University of Basel (Val d'Entremont, Val d'Anniviers and Val d'Hérens). Flora Europea nomenclature was used (Royal Botanic Garden Edinburgh, 2013). Voucher specimens of the plants are preserved at the Division of Pharmaceutical Biology in Basel, and at the Conservatory and Botanical Gardens of Geneva (CJB).

2.3. Data analysis and statistical evaluation

2.3.1. Classification

Already domesticated and commonly consumed food plants such as potatoes or fennel were excluded from the study. Botanical name, family, local name, parts used, and mode of utilization were retrieved for each plant species. The ecological characteristics of the food plants were also compiled, *i.e.* whether the species were cultivated (C) or collected in the wild (W). Classified as cultivated are those species which, according to Berlin's definition, are deliberately planted and managed by constant and direct intervention (Berlin, 1992). Food, drinks, and sweets or raw snacks were identified as the main categories of use, and eight subcategories were distinguished based on the traditional ways of use, including (1) salads, (2) cooked vegetables, (3) spices, (4) alcoholic drinks, (5) teas/coffee substitutes, (6) sirups, (7) jams, and (8) raw snacks (Table 2). Teas and alcoholic drinks were included even though they may be strictly considered rather as recreational products than as food. To be included, drinks should, however, not have been used for medicinal purposes, but as part of a diet.

2.3.2. Informants' consensus factor (F_{ic})

The Informants' Consensus Factor (F_{ic}) has been introduced by Trotter and Logan (Trotter and Logan, 1986; Heinrich et al., 2009) and is calculated as follows:

$$F_{ic} = \frac{n_{ur} - n_t}{n_{ur} - 1}$$

where n_{ur} is the number of use reports and n_t the number of species used, for each category.

This factor gives information about the consensus of informants for the consumption of a certain use-category (e.g. salads) and evaluates the variability of the mode of utilization for food plants (Heinrich et al., 1998, 2009). The product of this factor ranges from 0 to 1. A value close to 1 indicates that relatively few species are used by a large proportion of local people, while a low value indicates that the informants disagree on the species (Heinrich et al., 1998). It is assumed that the greater the independent citation of a particular species for a specific use-category, the greater is its cultural importance (Heinrich et al., 2009). A culturally important plant is therefore a species used by a large number of informants for the same category of indigenous use, while plants cited by only few people are considered to be of low cultural importance (Heinrich et al., 1998).

2.3.3. Cultural importance index (CI)

The cultural importance index is based on the informants' consensus factor and additionally takes into account the diversity of uses. It can be considered as a redefinition of the use-value of Phillips and Gentry (Phillips and Gentry, 1993), but regroups the information in a more practical way for the current study, *i.e.* by plants and use-categories (Tardio and Pardo-De-Santayana, 2008). The cultural importance index is the sum of all use-reports calculated for each category of use (UR_{ui}) concerning a specific plant species, divided by the total number of participants (N) (Tardio and Pardo-De-Santayana, 2008). It is calculated as follows:

$$CI_s = \sum_{u=u_1}^{u_{NC}} \sum_{i=i_1}^{i_N} \frac{UR_{ui}}{N}$$

This statistical value takes into account not only the spread of use (number of informants) for each species, but also the diversity of its uses. The CI index is an efficient tool for highlighting those species with a high-agreement for the whole survey area and, hence, to identify the shared knowledge (Tardio and Pardo-De-Santayana, 2008).

2.3.4. Use-report

Since an informant may mention several uses for a same species, the results should be presented together with the absolute use-reports, as exemplified by Giovannini and Heinrich (Giovannini and Heinrich, 2009; Heinrich et al., 2009). The use-report is defined as the number of informants that mention a particular species during the interviews (Giovannini and Heinrich, 2009).

2.4. Historical books

Two local libraries (Centre Régional d'Etude des Populations Alpines, CREPA in Sembrancher, and Hospice du Grand-Saint-Bernard, Bourg-St.-Pierre) were searched for relevant ethnobotanical literature. Information on food plants was extracted from historical books reporting ancient uses of plants in Valais.

Le guide du botaniste qui voyage dans le Valais was written by Laurent Joseph Murith, who was prior of the Congregation of the Grand-Saint-Bernard. It contains a precise description of the flora of Valais and some information on food plants (Murith, 1810).

Le Journal de pharmacie et des sciences accessoires was published by a commission of the Society of Pharmacy in Paris. Food traditions in the Alps including Valais are discussed (Bouillon-Lagrange et al., 1828).

La Phytographie médicale, histoire des substances héroïques et des poisons (Roques, 1835) and *Le Nouveau traité des plantes usuelles spécialement appliqué à la médicine domestique et au régime alimentaire* (Roques, 1837) were written by a French medical doctor and consist of monographs providing botanical data and information on uses of plants or detailed culinary preparations found in Valais, respectively.

Le *Glossaire du patois de la Suisse Romande* was written by a pastor of Montreux (canton of Vaud) and reported traditions of rural populations in Valais (Bridel and Favrat, 1866).

Les *Plantes médicinales indigènes ou cultivées en Valais. Leurs propriétés et emplois en médecine populaire*, written by a professor of botany in Sion, focused on forgotten traditional knowledge of Valais. It includes a lot of recipes and traditional uses of plants in Valais (Wolf, 1906).

Table 2
Identified edible plants consumed in Valais.

Family	Scientific name of the plant	Dialect	Used parts[a]	UR[b]	CI[c]	Ecosystem[d]	Salads	Cooked vegetables	Spices	Raw snacks	Alcoholic drinks	Teas	Syrups	Jams
Apiaceae	*Aegopodium podagraria* L.		Lv	3	0.2	W								
	Angelica sylvestris L.		Se	3	0.2	W								
	Carum carvi L.	Tsirié / Tsuryé	Se	13	0.7	W								
	Heracleum sphondylium L.	Plouta	ys, se	5	0.3	W								
	Levisticum officinale Koch		Lv	9	0.5	C								
	Peucedanum osthrutium (L.) Koch	Utrëhle	Rt/Fl/Se	8	0.4	W								
Aspleniaceae	*Asplenium trichomanes* L.			2	0.1	W								
Asteraceae	*Achillea moschata* Wulfen			4	0.2	W								
	Achillea millefolium L.	Erba di tsapwï	Aer	10	0.5	W								
	Artemisia dracunculus L.		Lv	1	0.1	C								
	Artemisia genipi G. Weber	Dzinepé / Pòhima	Lv	12	0.6	W								
	Bellis perennis L.	Marderita	Fl	1	0.1	W								
	Calendula officinalis L.		Fl	1	0.1	C								
	Carlina acaulis L.	Tsardon	Fl/Ro	16	0.8	W								
	Cichorium intybus L.			3	0.0	C								
	Cirsium spinosissimum (L.) Scop.	Tsardon	Fl	6	0.3	W								
	Leontopodium alpinum Cass.	èlveis / pyà de tsà	Fl	3	0.2	C								
	Tanacetum balsamita L.		Fl/Aer	1	0.1	W								
	Taraxacum officinale G. Weber	Poùpa / Troyachè	Lv/Fl	38	1.3	W								
	Tragopogon pratensis L.	barbaoù doeu	Lv/ys	8	0.4	W								
	Tussilago farfara L.	Taconnet	Fl	10	0.5	W								
Berberidaceae	*Berberis vulgaris* L.	Boson de rodzëta	Ber	5	0.3	W								
Betulaceae	*Betula pubescens* L.	Byöla / byöa	sap	1	0.0	W								
Boraginaceae	*Borago officinalis* L.	Bòràtse	Fl	5	0.3	W								
	Symphytum officinale L.	Konsolida	Lv/Rt	4	0.2	W								
Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.	Erba di poùte	Aer	3	0.2	W								
	Cardamine pratensis L.		Aer	1	0.1	W								
	Nasturtium officinale R. Br.	Krèson	Aer	6	0.3	W								
Campanulaceae	*Campanula rapunculus* L.	Kanpanïn	Ros	2	0.1	W								
	Phyteuma orbiculare L.		Fl/Ros	2	0.1	W								
	Phyteuma spicatum L.		Fl/Ros	1	0.1	W								
Caprifoliaceae	*Sambucus nigra* L.	Syoeu né / Chaouk	Ber/Fl	29	0.9	W								
	Sambucus racemosa L.	Syoeu ródzõ	Ber/Fl	5	0.1	W								
Caryophyllaceae	*Saponaria officinalis* L.		Aer	1	0.0	W								
	Silene cucubalus Wibel	hlõtë / hlök	Lv	2	0.1	W								
Chenopodiaceae	*Chenopodium bonus-henricus* L.	vèrkouènyo	Lv	26	1.4	W								
	Chenopodium album L.		Lv	2	0.1	W								
Cornaceae	*Cornus mas* L.		Ber	1	0.0	W								

To be continued...

3. Results

During our ethnobotanical investigations, a total of 707 answers were recorded concerning the use of 98 edible plants. Three main groups and eight subcategories of uses could be identified with regard to the mode of consumption (Table 1): (1) plants used as drinks, including teas or coffee substitutes, alcoholic drinks, and sirups; (2) alimentary plants being processed, such as cooked vegetables, salads, and spices; (3) plants eaten as raw snacks or sweets (jams).

Wild species represent about 87% of the whole recorded food species in this study. The plant parts most commonly consumed as food were flowers (26%), leaves (21%), complete aerial parts (18%), and fruits (18%) Table 2.

The categories with the highest number of mentions were teas (18%), followed by cooked vegetables (16%), jams (16%), and raw snacks (16%) (Fig. 1).

Informant's consensus between 0.59 and 0.82 were obtained for the different food use categories. The category with the highest F_{ic} was jams (0.82) followed by cooked vegetables (0.77). The species responsible for the high consensus of jams were *Taraxacum officinale* and *Vaccinium myrtillus*, with 16 citations each in this use category. For the utilization as cooked vegetables, the most cited species were *Chenopodium bonus-henricus* and *Urtica dioica*, with

Table 2 (*continued*)

Family	Scientific name of the plant	Dialect	Used parts[a]	UR[b]	CI[c]	Ecosystem[d]	Salads	Cooked vegetables	Spices	Raw snacks	Alcoholic drinks	Teas	Syrups	Jams
Cupressaceae	*Juniperus communis* L.	dzenièvre	Ber	12	0.3	W								
Equisetaceae	*Equisetum arvense* L.	Kawëta	Aer	8	0.2	W								
Ericaceae	*Vaccinium myrtillus* L.	Outrille	Ber/Lv	19	0.7	W								
	Vaccinium vitis-idaea L.	Gravèlòng	Ber	13	0.3	W								
Fabaceae	*Astragalus cicer* L.		Fr	1	0.0	W								
	Robinia pseudoacacia		Fl	2	0.1	W								
	Medicago sativa L.	sanfwïn	Fl	1	0.0	W								
	Trifolium alpinum L.		Fl	2	0.1	W								
	Trifolium sp.		Fl	5	0.1	W								
	Vicia faba L.	fáva	Fr	1	0.0	C								
Fagaceae	*Quercus* sp.	tsanyõ	Bark	1	0.0	W								
Gentianaceae	*Gentiana lutea* L.	Einsangna	Rt	9	0.2	W								
	Gentiana verna L.	Einsangna	Aer	1	0.0	W								
Hypericaceae	*Hypericum perforatum* L.	Millèpèrtouitt	Fl	3	0.1	W								
Lamiaceae	*Lavendula officinalis* Miller		Aer	1	0.0	C								
	Melissa officinalis L.	Melïsa	Lv	3	0.1	C								
	Origanum vulgare L.		Lv	6	0.2	C								
	Salvia pratensis L.	Bonómo blu	Lv	3	0.1	W								
	Satureja acinos (L.) Scheele		Aer	5	0.1	W								
	Satureja montana L.		Aer	1	0.0	W								
	Thymus pulegioides L.		Aer	5	0.1	C								
	Thymus serpyllum L.	Pïnpyõlë	Aer	9	0.3	W								
Liliaceae	*Allium ursinum* L.	de zo / ale	Lv	14	0.4	W								
	Allium Schoenoprasum L.		Lv	3	0.1	W								
Malvaceae	*Malva* sp.	motëta / mârya	Fl	4	0.1	C								
Onagraceae	*Epilobium parviflorum* Schreber	Boùnyë di tsyöre	Fl	3	0.1	W								
Oxalidaceae	*Oxalis acetosella* L.	Pan de koùtu	Lv	16	0.4	W								
Papaveraceae	*Chelidonium majus* L.	èrba di vèrouyè	Aer	1	0.0	W								
Parmeliaceae	*Cetraria islandica* (L.) Ach	Mourà della yèd	Tha	2	0.0	W								
Pinaceae	*Picea abies* (L.) Karsten	Vagne / sapïn	Bd/Rs	29	0.8	W								
	Pinus cembra L.	moungnètt	Se	1	0.0	W								
Plantaginaceae	*Plantago lanceolata* L.	Plangtarl lòng	Lv	9	0.3	W								
	Plantago media L.	Plangtarl platt	Lv	4	0.2	W								
	Euphrasia sp.	Tartari du reko	Aer	2	0.1	W								
	Verbascum sp.	Bonómo dzónõ	Lv/Fl	2	0.1	W								
Polygonaceae	*Rumex acetosa* L.	barbaoù	St/Lv	16	0.5	W								
	Rumex alpinus L.	Lapi / Làpé	St	9	0.3	W								
Polypodiaceae	*Polypodium vulgare* L.	ri de galise di krepon	Rh	11	0.3	W								
Primulaceae	*Primula vulgaris* Huds.	Mei de mé	Fl	3	0.1	W								
	Primula veris Huds.	Mei de mé	Fl	6	0.2	W								
Rosaceae	*Agrimonia eupatoria* L.		Aer	3	0.0	W								
	Alchemilla alpina L.	Pòrta-rozo	Aer	10	0.3	W								
	Alchemilla xanthochlora aggr.	Porta-rozo	Aer	10	0.3	W								

To be continued...

25 and 27 citations, respectively. The category of alcoholic drinks showed the lowest consensus factor (0.59) (Table 3).

During this ethnobotanical survey, teas always represented the most frequently reported use of food plants (18%), and the most cited use within drinks (61%), although little consensus was found between informants with regard to the species to be used (F_{ic}=0.66). *Alchemilla sp.* (CI=0.27) was the most cited plant for tea in Val d'Anniviers, Val d'Hérens and Val d'Entremont, but was not really used in Val d'Illiez. Informants told that this tea could be considered as a women's tea, because of its properties against premenstrual pains and menopausal problems. On the other hand, a relatively small number of plants (5%) were used to prepare sirups. Informants in Entremont made sirups from young buds of *Picea abies*, and from flowers or fruits of *Sambucus nigra*. In Val d'Anniviers, diverse Lamiaceae (*Salvia*, *Thymus*, etc.) were added to enhance taste and curative properties of different sirups.

Salads and cooked vegetables represented 11% and 16% of the cited uses of food plants, respectively. These plants were a considerable part of the daily diet during the spring season. The young shoots of the bitter dandelion (*Taraxacum officinale*) (CI=1.3) are still commonly eaten as a salad (CI_{salad}=0.62) mixed with rampion, eggs and fried bread. Nettle (*Urtica dioica*) (CI=0.8) was eaten as a soup (CI_{soup}=0.71). Stems of some young plants were collected (e.g. *Tragopogon pratensis*, *Heracleum sphondylium*, *Taraxacum officinale*). Petals (*Calendula officinalis*, *Tropaelum majus*, *Borago officinalis*, *Phyteuma orbiculare*) or spices (*Carum carvi*)

Table 2 (*continued*)

Family	Scientific name of the plant	Dialect	Used parts[a]	UR[b]	CI[c]	Ecosystem[d]	Salads	Cooked vegetables	Spices	Raw snacks	Alcoholic drinks	Teas	Syrups
Rosaceae	*Crataegus monogyna Jacq.*	Flò dë sinbu	Fl	3	0.1	W							
	Filipendula ulmaria (L.) Maxim.		Fl	3	0.1	W							
	Fragaria vesca L.		Fr	10	0.4	W							
	Malus sylvestris Miller		Fr	1	0.1	W							
	Prunus avium L.	Siryizyï	Ber	3	0.1	W							
	Prunus spinosa L.	Belosai	Ber	4	0.2	W							
	Rosa canina L.	Grata-tu	Ber	10	0.3	W							
	Rosa sp.		Fl	1	0.0	C							
	Rubus idaeus L.		Fr	14	0.4	W							
	Sanguisorba minor Scop.		Lv	2	0.1	W							
	Sorbus aria (L.) Crantz	Alizé	Ber	8	0.2	W							
	Sorbus aucuparia L.		Ber	2	0.1	W							
Rubiaceae	*Galium odoratum* (L.) Scop.		Aer	2	0.1	W							
Tiliaceae	*Tilia cordata* Miller	Tiyoél	Fl	2	0.1	W							
Tropaeolaceae	*Tropaeolum majus* L.		Lv/Fl	5	0.1	C							
Urticaceae	*Urtica dioica* L.	Oùrtyà	Lv	27	0.8	W							
Violaceae	*Viola tricolor* L.	Vyöta dzóna	Fl	4	0.1	W							

[a] Used parts: Lv-leaf; Aer-aerial part; Rh-rhizome; Rs-rosette; Fl-flower; Fr-fruit; Ber-berry; Rt-root; Rs-resin; Ys-young stem; St-stem; Se-seed; Th-thallus; Bd-bud. [b] UR-Use report. [c] CI-cultural importance index calculated from the formula: $CI = \sum_{u=u_1}^{u_{NC}} \sum_{i=i_1}^{i_N} \frac{UR_{ui}}{N}$. [d] Ecosystem: W-wild; C-cultivated.

Mention of use: Green – Val d'Entremont; Red - Val d'Illiez; Orange - Val d'Anniviers; Blue - Val d'Hérens; Brown - Ancient treaties.

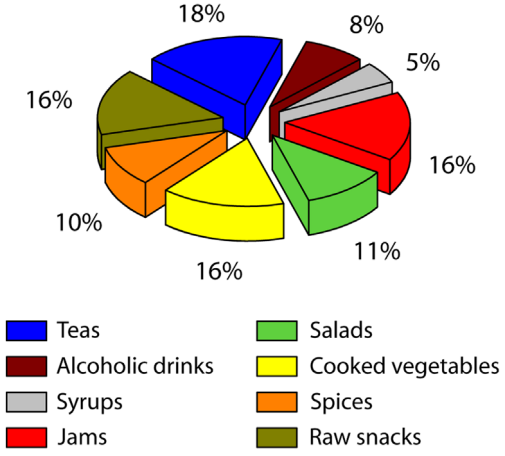

Fig. 1. Modes of utilization. Percentages correspond to the ratio use report of each mode of utilization (n_{UR}) divided by the total of all use reports (707) in the study.

Table 3
Frequency of use for each category.

Category of indigenous use		Number of species (n_s)	Number of use-reports (n_{UR})	Informants' consensus factor (F_{ic})
Food	Salads	30	79	0.63
	Cooked vegetables	27	113	0.77
	Spices	20	69	0.72
Drinks	Teas	41	128	0.69
	Alcoholic drinks	24	57	0.59
	Sirups	12	36	0.69
Snacks and sweets	Jams	22	116	0.82
	Raw snacks	28	112	0.76

were sometimes added for gustatory or decorative purposes. The latter use may, however, be of a more recent origin. A kind of pesto was prepared from leaves of wild garlic (*Allium ursinum*) which grows abundantly in the region.

Numerous traditional plants (16%) were consumed as snacks. This high proportion can be explained by the long time that children spent in the fields and pastures. As soon as the herbage had sufficiently grown in the high mountain pastures, peasants used to move from the villages to their *mayen*. They spent the summertime there producing cheese and pasturing. Children served as shepherds, and elder children showed the younger ones which herbs to eat as snacks. Some plants, such as *Carlina acaulis*, *Cirsium spinosissimum* were eaten like artichokes, by removing the surrounding leaves to reach the edible receptacles. Young shepherds from Val d'Entremont sucked some sweet petals (*Trifolium pratense*, *Trifolium alpinum* – the liqueurice of the Alps-, *Phyteuma orbiculare*), sweet stems (*Polygonum bistorta*), sweet rhizomes (*Polypodium vulgare* – the liqueurice of the woods), and gum of

Picea abies and *Larix decidua*. They also appreciated sour herbs, such as *Oxalis acetosella*, *Rumex acetosa*, and leaves of *Vaccinium myrtillus*, and spicy plants like *Nasturtium officinale*.

Jams were produced from different fruits (*Vaccinium myrtillus*, *V. vitis-idaea*, *Ribes sp.*, *Berberis vulgaris*, *Rosa canina*) and from flowers (*Taraxacum officinale*). The informant of Val d'Hérens told that she prepared sweet candies with *Angelica sylvestris*.

Taraxacum officinale was the culturally most important plant according to the CI index (CI=1.34) and was cited by all informants (UR=38). Its uses as salad (CI_{salad}=0.62) or as jam (CI_{jam}=0.43) were known in all valleys (see Table 2). The second species in the ranking was *Sambucus nigra* (CI=0.95), which is collected for the confection of jams (CI_{jam}=0.29) mostly in Val d'Illiez, or for preparation of sirups (CI_{syrup}=0.32) in Val d'Entremont. High cultural importance indices were also found for *Urtica dioica* (CI=0.84), *Picea abies* (CI=0.79), and *Vaccinium myrtillus* (CI=0.74). Interestingly, most plants with high CI score were utilized in all investigated regions (Table 2).

In order to identify further plants which may have already fallen in oblivion, several books published in the XIXth or early XXth century were consulted. These books had been written by botanists, medical doctors, pharmacists or clergy, and proved to be a rich source of information on plants used during the last century in canton of Valais. Some authors had at that time already the impression that knowledge was getting lost and, hence, wanted to document traditional uses. For all categories of uses, except jams, some plants are reported which were no more cited by the informants. In addition, there are also plants such as *Vaccinium vitis-ideae* or *Fragaria vesca* which are still consumed, but no more for a particular mode of use (Table 4).

While therapeutic aspects were not the primary focus of our survey, a strong association was observed between the food and medicinal uses of plants. Most informants mentioned that several food plants were consumed also for health purposes. In spring, inhabitants of the canton of Valais are used to eat wild bitter salads (*Taraxacum officinale*, *Allium ursinum*), soups (*Chenopodium bonus-henricus*, *Urtica dioica*) or drinks (birch sap) reputed to be depurative. These plants are believed to facilitate digestion and "to purify the blood". They are reputed for their high contents in minerals. *Taraxacum officinale* is known to possess high content in potassium conferring diuretic properties to the plant, whereas *Chenopodium bonus-henricus* and *Urtica dioica* are believed to be rich in iron and, therefore, considered to reduce tiredness.

In autumn, some informants eat powdered roots of *Gentiana lutea* and leaves of *Vaccinium myrtillus* as preparation for the cold season. Many plants are utilized against cough or colds (28 species). These plants are often consumed as teas and include plants of the Lamiaceae family rich in antimicrobial monoterpenes, and species containing mucilages (Plantago spp, *Malva* spp., *Cetraria islandica*, *Tussilago farfara*, *Verbascum* spp.). The use of *Plantago* spp. and *Peucedanum ostruthium* as teas or fumigations appears widespread in Valais.

Plants reported to have beneficial effects on digestive functions also represent an important group (14 species against flatulence, 2 against diarrhea, 3 against constipation). Inhabitants used to drink gentian schnapps as a digestive after their meal. A complete list of plants cited during the interviews for various therapeutic indications is provided in Table 5, and detailed information with regard to the use in the different valleys is available in Appendix A.

4. Discussion

4.1. Wild plants traditionally consumed by the population of Valais

4.1.1. Teas and coffee substitutes

Switzerland possesses a long tradition with regard to the preparation of teas. In the XVIIIth and XIXth century, the Swiss tea was a famous specialty prepared with a mixture of alpine plants. Wolf and Roques cited as ingredients *Tussilago farfara*, *Thymus serpyllum*, *Salvia sp.*, *Filipendula ulmaria*, *Achillea sp.*, *Origanum vulgare*, *Hyssopus officinalis*, *Stachys officinalis*, *Teucrium chamaedrys*, *Rosmarinus officinalis*, *Gnaphalium dioicum*, *Scabiosa lucida*, *Asperula odorata*, *Glechoma hederacea*, *Arnica montana*, and *Tanacetum alpinum* (Roques, 1837; Wolf, 1906). The French philosopher Jean-Jacques Rousseau (1712–1778) mentioned this Swiss tea recipe in his book entitled *Les Confessions* (Rousseau, 1825). This tea was a substitute for the expensive teas imported from Asia (Künzle, 1914). It constituted a substantial income for inhabitants of alpine cantons (Flückiger, 2000), and was served in some mountain restaurants of Valais (Töppfer, 1901). Further plants, such as *Dryas octopetala* or *Veronica officinalis* were added as ingredients giving the "Tea of Professors" and "European Tea", respectively (Wolf, 1906; Künzle, 1914). Not only black tea was out of reach for poor peasants, but also coffee. Inhabitants of the Valais

Table 4
Historical uses of food plants which were no more cited by informants.

	Use	Forgotten plants
Food	Salads	*Sonchus alpinus*[b], *Barbarea praecox*[a], *Lepidium sativum*[a].
	Cooked vegetables	*Borago officinalis*[a], *Sonchus alpinus*[b], *Carlina acanthifolia*[b].
	Spices	*Glechoma hederacea*[a], *Marrubium vulgare*[a], *Asplenium ruta muraria*[a].
Drinks	Teas	*Achillea nana*[a], *Arnica montana*[a], *Gnaphalium dioicum*[a], *Tanacetum alpinum*[b], *Vaccinium vitis-ideae*[a], *Glechoma hederacea*[a], *Origanum vulgare*[a], *Stachys officinalis*[a], *Teucrium chamaedrys*[a], *Dryas octopetala*[d], *Veronica officinalis*[a,d], *Quercus petrae*[a].
	Alcoholic drinks	*Foeniculum vulgare*[a], *Achillea atrata* [a,b], *Achillea nana*[a], *Artemisia glacialis* [a,c], *Artemisia mutellina*[c], *Artemisia rupestris*[c], *Artemisia vallesiaca*[b,c], *Vaccinium vitis-idaea*[a], *Hypericum perforatum*[a], *Glechoma hederacea*[a], *Physalis alkekengi*[a], *Fragaria vesca*[a], *Angelica sylvestris*[a], *Athamanta cretensis*[b], *Coriandrum sativum*[a].
	Sirups	*Berberis vulgaris*[a], *Rhamnus cathartica*[a], *Physalis alkekengi*[a], *Nasturtium officinale*[a].
Snacks and sweets	Jams	–
	Raw snacks	*Tropaeolum majus*[a], *Physalis alkekengi*[a], *Prunus spinosa*[a,c], *Carlina acanthifolia*[e], *Fagus sylvatica*[f], *Bunium bulbocastanum*[e].

[a] (Wolf, 1906).
[b] (Roques, 1837).
[c] (Bouillon-Lagrange et al., 1828).
[d] (Künzle, 1914).
[e] (Boissier, 1897).
[f] (Bridel and Favrat, 1866).

Table 5
Medicinal uses of food plants cited by informants.

Therapeutic area	Plants[a]
Cardiovascular system	*Levisticum officinale* (a), *Taraxacum officinale* (a), *Betula pubescens* (a), *Saponaria officinalis* (a), *Chenopodium bonus-henricus* (a), *Juniperus communis* (a), *Equisetum arvense* (a), *Allium ursinum* (a), *Primula veris* (a), *Filipendula ulmaria* (a), *Galium odoratum* (a), *Urtica dioica* (a), *Viola tricolor* (a), *Capsella bursa-pastoris* (b), Crataegus monogyna (b,c)
Endocrine system	*Achillea moschata* (d,e), *A. millefolium* (d,e), *Alchemilla alpina* (d,e), *A. xanthochlora agg.* (d,e), *Salvia pratensis* (e), *Gentiana lutea* (f), *Vaccinium myrtillus* (f), *Cichorium intybus* (g)
Gastrointestinal system	*Carum carvi* (h,i), *Levisticum officinale* (h), *Peucedanum osthrutium* (h), *Achillea moschata* (h), *A. millefolium* (h), *Vaccinium myrtillus* (h,i,j), *Gentiana lutea* (h,k), *G. verna* (h,i), *Hypericum perforatum* (h), *Salvia pratensis* (h), *Thymus pulegioides* (h), *T. serpyllum* (h), *Malva sp.* (h), *Alchemilla xanthochlora agg.* (h), *Sorbus aucuparia* (h), *Urtica dioica* (h), *Leontopodium alpinum* (j), *Rosa spp.* (j)
ORL and respiratory system	*Heracleum sphondyllium* (l), *Artemisia genipi* (l,n), *Leontopodium alpinum* (l), *Tussilago farfara* (l,n), *Borago officinalis* (l,n), *Sambucus nigra* (l,m,n), *Juniperus communis* (l), *Hypericum perforatum* (l), *Melissa officinalis* (l), *Origanum vulgare* (l), *Thymus pulegioides* (l,o), *T. serpyllum* (l,n,o), Malva sp. (l), *Cetraria islandica* (l,m), *Picea abies* (l,n), *Plantago lanceolata* (l,m,n), *P. media* (l,n), *Verbascum sp.* (l,n), *Polypodium vulgare* (l), *Primula veris* (l,n), *Tilia cordata* (l,n), *Viola tricolor* (l,n), *Achillea moschata* (n), *A. millefolium* (n), *Levisticum officinale* (n), *Peucedanum osthrutium* (n) *Salvia pratensis* (o)
Hematopoiesis	*Chenopodium bonus-henricus* (p), *Rosa canina* (p), *Rumex alpinus* (p), *Plantago lanceolata* (q)
Pain	*Peucedanum osthrutium* (r), *Thymus pulegioides* (r), *Verbascum sp.* (r), *Filipendula ulmaria* (s), *Urtica dioica* (s), *Malva sp.* (s), *Juniperus communis* (s), *Sambucus nigra* (s)
Neurologic system	*Hypericum performatum* (t), *Lavendula officinalis* (u), *Origanum vulgare* (t), *Melissa officinalis* (u), *Malva sp.* (u), *Tilia cordata* (u), *Galium odoratum* (u), *Sorbus aucuparia* (u), *Agrimonia eupatoria* (u).
Urogenital system	*Equisetum arvense* (v), *Prunus avium* (v), *Artemisia genipi* (w), *Rumex alpinus* (w)

[a] Indications: (a): Depurative, diuretic; (b): cardiostimulant; (c): kidney stones; (d) against menstruation disorders; (e) during menopause; (f): antidiabetic; (g): hepatoprotective; (h): antiflatulent, antibloating, against colic; (i): laxative; (j): antidiarrheal; (k): appetite giver; (l): antitussive, against bronchitis; (m): antiallergic; (n): against cold; (o): sore throat; (p): antianemic; (q): immunostimulant; (r): against headache; (s): antirheumatic; (t): antidepressor; (u): sleep inducer; (v): urinary desinfectant; (w): aphrodisiac.

were therefore used to mix coffee with other plants, such as chicory (*Cichorium intybus*, Asteraceae) or beech (*Fagus sp*., Fagaceae). Seeds or roots were dried and roasted in a pot, then ground and prepared as decoctions. Increasing accessibility of coffee led to a progressive abandoning of these substitutes in the mid-XXth century.

4.1.2. Alcoholic drinks

Production of distilled alcoholic beverages is still popular in canton of Valais. The consumption of schnapps and liqueurs decreased, however, during the XXth century: in 1900, each inhabitant of Switzerland drank 3 L of such drinks, whereas the consumption had dropped to less than 2 L in 2008 (RFA, 2012). The introduction of restrictive Swiss legislation on alcohol in the years 1880–1940 was one reason for this. The introduction of a license for households and higher prices aimed to diminish home production (Hercod, 1930). These measures to protect the Swiss population from alcoholism probably contributed to reduce the consumption of traditional brandies. Nevertheless, spirits are still present as traditional drinks as revealed by our study. Two popular alcoholic drinks in Valais are the *genépi* (liqueur and brandy), and the *gentiane* (brandy). Both of these plants have a long tradition in Valais.

Genepi, mentioned in ancient treaties, was called under several names depending on the exact species used: black genepi or men's genepi (*Artemisia genepi*), white genepi or women's genepi (*Artemisia umbelliformis*) (Nicollier and Nicollier, 1984). J.J. Virey praised the higher quality of the very aromatic liqueur coming from *Artemisia glacialis* or *Artemisia vallesiaca* (Bouillon-Lagrange et al., 1828). Some other writers reported that genepi was prepared from various species of *Achillea* (*A nana*, *A. moschata*, *A. atrata*) (Mérat, 1831). Interestingly, interviews in Val d'Entremont and Val d'Anniviers revealed that the liqueur is today made from *Artemisia genipi or A. umbelliformis* (nowadays domesticated). *Artemisia glacialis* and *Artemisia vallesiaca* are rare species and thus should not be used any more for preparation of liqueurs. Informants in Entremont recalled to have tasted a liqueur containing *Achillea moschata*, which grows abundantly in the region. This species is often used to prepare genepi liqueur in the nearby Vallée d'Aoste (Italy).

The production of gentian schnapps from the roots of *Gentiana lutea* seemed to be an old tradition, since it was already mentioned in an ancient book (Roques, 1837). This beverage was formerly considered as a preparation helping peasants to digest their rather fatty food. It was particularly popular in Val d'Entremont (8 citations) where yellow gentian is common, but surprisingly was not cited in Val d'Illiez. Inhabitants used to collect the roots of the *Gentiana lutea* in autumn. In Val d'Anniviers, where this species is rarer, people used the roots of *Gentiana purpure*a instead. This related species is common in the region (Brüschweiler, 1999).

People consume nowadays mainly schnapps prepared by distillation of fruits (pears William, apricot). The schnapps may be added to coffee (*café arrosé*), or is taken as digestive aid after a meal. Although most spirits are nowadays produced at industrial scale, interviews revealed that local people continue to prepare alcoholic drinks from wild plants.

4.1.3. Salads and cooked vegetables

Plants growing in nitrogen-rich biotopes (*Rumex sp*., *Chenopodium bonus-henricus*, *Urtica dioica*) were particularly attractive as vegetables. These plants are characteristic and abundant in the nitrogen-rich pastures in proximity of the “mayens” and stables. They were eaten as soups, as boiled vegetables, or salads. For example, in Val d'Anniviers, informants cooked *La soupe du Mayen* (soup of the chalet) which contained *Urtica dioica*, *Taraxacum officinale*, *Chenopodium bonus-henricus*, *Allium sativum*, and *Levisticum officinale*. In Val d'Illiez during the Second World War, informants reported that some families fought against each other to collect *Rumex alpinus,* a wild rhubarb. Buds of flowers of *Heracleum sphondylium* were also cooked as vegetables.

4.1.4. Snacks

For a part of informants, many of the reported uses existed only in the collective memory. Most wild fruits, bulbs or flowers mentioned in this study were consumed in childhood as snacks or for amusement when tending livestock. Some elders still pick them on walks and show them to grandchildren to relive the

flavors of their childhood. Some informants told that they tried any kind of herbs and fruits just by curiosity. Other inhabitants, in contrast, indicated that they were not allowed to taste berries they could not identify with certainty. The recreational consumption of snacks during agricultural activities is in line with observations made in other European countries (Luczaj et al., 2012). Several studies reported that flowers (e.g. *Lamium album*), mature fleshy fruits (e.g. *Vaccinium myrtillus*), nuts, seeds or immature fruits (e.g. *Capsella bursa-pastoris*) were often consumed as snacks (Soukand and Kalle, 2013; Luczaj et al., 2012). Like in other countries, most of the plants eaten by children in Valais were sweet (fruits, flower nectar) or sour (e.g. *Rumex* spp., *Oxalis* spp., *Berberis vulgaris*).

4.2. Transmission of the knowledge and use of the plants nowadays

Elderly informants reported that cultural knowledge was transmitted orally, "vertically", from the older generation to the younger ones, or within the same generation from elder people. Vertical transmission has been found to be important during the interviews of younger inhabitants ($n=4$, less than 40 years). In addition, local inhabitants commonly used reference books. The most consulted books were written by Cousin, Kneipp, Künzle, Treben, and Couplan (Künzle, 1914; Treben, 1998; Cousin-Zermatten, 2005; Couplan, 2009; Cousin-Zermatten, 2009, 2010). The globalization of our society influenced also local communities and resulted in the introduction of "new" plant species, and knowledge through the media. Birch sap (*Betula pubescens*) is a typical example of a plant product which seems to have been introduced only recently and was not mentioned by informants older than 70. Originating from Eastern Oriental tradition, it was adopted by the young generation in Valais as a depurative, and as a treatment for rheumatic diseases. Knowledge is found essentially in the older generation. As a consequence of societal changes and urbanization the younger generation is much less familiar with the traditional uses of wild plants. Also, modifications of the habitats have led to a decrease in abundance of some species (e.g. *Polypodium vulgare* and *Nasturtium officinale* in Val d'Entremont). The fear of catching echinococcosis, an incurable parasitary disease transmitted by fox feces, also keeps some people from gathering food plants, such as wild berries (e.g. *Vaccinium myrtillus*, *Fragaria vesca*).

Some traditional uses reported in ancient treaties were no more mentioned by the informants. The reasons can be diverse. Some plants may still be used in other valleys of Valais (particularly in the South of Alps) or their use may be only occasional and not known by our informants. In some cases, cultivated species have obviously supplanted wild plants. For example, the liqueur of wild strawberry was replaced by products obtained from widely cultivated varieties. Certain plants, such as *Bunium bulbocastanum*, were recognized by none of our informants and appear to have fully disappeared from the traditional knowledge.

4.3. Possible toxicity

The plants cited during the interviews appear devoid of acute toxicity. However, it must be critically noticed that some species contain secondary metabolites of toxicological relevance. For example, pyrrolizidine alkaloids are known to be hepatic and pulmonary toxicants, and to possess high antimitotic and genotoxic activity *in vitro* and *in vivo* (Petry et al., 1984; Kim et al., 1993). Well studied pyrrolizidines include senkirkine contained in *Tussilago farfara*, symphitine from *Symphytum officinalis*, and lycopsamine found in *Borago officinalis*. During the interviews in Val d'Entremont, some people were conscious of the toxicity of these plants but conceded that they occasionally used them nevertheless. In Val d'Hérens, the informant recommended to blanch *Tussilago farfara* before cooking it as gratin. The effectiveness of such procedures, however, is not known.

Linear furanocoumarins contained in *Heracleum sphondylium*, *Levisticum officinalis*, or *Angelica sylvestris* may cause photosensitization and possess carcinogenic properties (Diawara et al., 1995). On the basis of the risk assessment of dietary furanocoumarins made by Swiss authorities, the average daily intake of dietary furanocoumarins has been estimated to be of 1.45 mg, with high-exposure peak values of up to 14 mg (Schlatter et al., 1991; Deutsche Forschungsgemeinschaft (DFG), 2012). These evaluations have come to the conclusion that the risk arising from dietary furanocoumarins in a normal diet is very small or insignificant. However, the consumption of wild plants containing high level of these substances may be toxicologically relevant. Consequently, sensitive groups such as children and pregnant women should avoid consumption such plants (Committee on Herbal Medicinal Products (HMPC), 2007).

Plants of the genus *Oxalis*, *Rumex* and *Chenopodium* are known to be rich in oxalic acid, which may lead to hypocalcaemia and formation of calcium oxalate crystals in kidneys (Luczaj, 2010). *Arnica montana*, which contains the toxic sesquiterpene lactone helenaline, was part of the recipe for Swiss Tea (Wolf, 1906). However, none of our informants used this plant for oral preparations.

This study should by no means encourage inexperienced persons to gather wild plants. Around 2000 to 3000 persons required help after accidental plants ingestion and 26 intoxication cases with moderate to severe symptoms were reported in 2012 (Tox-Zentrum, 2012). Intoxications are most often due to confusion of morphologically similar species, e.g. between *Digitalis* and plants used as salads, leaves of *Atropa belladonna* and those of *Amaranthus spp*. (salads), leaves of *Colchicum autumnale* and those of *Allium ursinum* (pesto), or between *Veratrum album* and *Gentiana lutea* (schnapps). Safe collection of wild plants requires sound knowledge of botany. Self-learning through media or internet is probably not sufficient and can lead to severe intoxications.

4.4. Comparison with other studies in alpine areas

The culinary tradition of Valais is close to that of the Tyrolean region in Austria. The cited food plants and their uses are similar (Christanell et al., 2010). Some regional differences are, however, apparent: *Achillea* collected in Valais is from the species *Achillea moschata* which grows abundantly in region of Val d'Entremont and possesses a more intense smell than *Achillea millefolium* more commonly used in other countries (Wolf, 1906). People native to Valais share traditions with inhabitants of Northern Italy (Piemont, Aosta). Examples include the preparation of genepi schnapps, uses of similar snacks (receptacles of *Carlina acaulis*), and frequent use of *Taraxacum officinale* as salad (Armand, 1993; Pieroni and Giusti, 2009). These similarities are not surprising given the geographical proximity, similar dialects of Val d'Entremont and Val d'Aosta, and historically strong economic ties.

Other studies in Europe indicated that gathering of plants was usually regarded as an activity for women (Pieroni, 1999; Ertug, 2000; Christanell et al., 2010). In Valais, this activity appears more equally distributed. The confection of schnapps or liqueurs was a male activity, whereas women usually took care of collecting plants to be used as salads, vegetables or teas (Perrenoud and Roguet, 1999).

5. Conclusions

Like other rural areas in Europe, the Valais had a rich tradition of wild plant consumption. However, the modernization of rural societies in the Swiss Alps was characterized by a shift from

subsistence farming to a market oriented economy and considerable improvements in healthcare resulted in a major change of attitude in the local population. Historically, wild food plants were part of the diet and were of critical importance in times of food scarcity. Meanwhile, they have lost their role as vital components of the diet, and are nowadays perceived and appreciated as delicacies (Roguet, 2004).

Cultivation of alpine plants represents a new market niche for mountain agriculture (Fournier, 2003). More than 100 medicinal and aromatic plants have been taken into cultivation during the last three decades (Agridea, 2010). 350 ton of alpine herbs produced in Valais are being sold every year for the production of sweets, spices, drinks and cosmetics (Agridea, 2010). In this context, the numerous edible wild plants identified in the course of this study could provide interesting opportunities for further diversification of mountain agriculture.

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Appendix A. Supplementary materials

Supplementary materials associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.11.022.

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Appendix

Ethnobotanical survey on wild alpine food plants in Lower and Central Valais (Switzerland)

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Table of content

Appendix A. Questionnaire Abbet.

Summary of the questionnaire

Formal information: name, date of birth, place of birth, address, phone number, source of the plant knowledge (tradition, media…).

For each plant, can you mention the used part (leaves, flowers, roots), the mode of cooking and the possible therapeutic properties?

1. Can you mention any forgotten vegetable or spice?
2. Which plants do you use as:
    - **a.** teas
    - **b.** liqueurs/brandies
    - **c.** soups or cooked vegetables
    - **d.** salads
    - **e.** spices
    - **f.** jams
    - **g.** sirups
    - **h.** Other uses
3. Can you mention any diet involving wild plants?
4. Which plants do you use for your diet?
5. Which plants do you collect by yourself?

Do you know other people I can contact?

Christian Abbet

Route de Podemainge

1937 Orsières

Questionnaire sur les plantes comestibles

UNI BASEL

Questions

Année de naissance : _____

D'où vient votre savoir ? : _____

De quelle région venez-vous vous-même (enfance) ? :

Valais (district) : _____

Autre canton : _____

Où habitez-vous maintenant ? : _____

Pouvez-vous mentionner pour chaque plante la partie utilisée (feuilles, fleurs, racines), la préparation (cuit dans l'eau, mangé cru, distillé, sous forme d'infusion) et les propriétés thérapeutiques éventuelles.

1. Connaissez-vous des légumes ou des épices qui ne sont plus utilisés aujourd'hui?

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

2. Selon vous, quelles plantes sauvages sont ou ont été utilisées:

En tant que thés

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

pour faire des liqueurs

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

En tant que soupes ou légumes

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

En tant que salades

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

en tant qu’épices (salaison, fromage aux herbes, beurre aux herbes…)

Pflanze	Benutzter Teil	Vorbereitung	Heilwirkung

Pour faire des confitures, des gelées ou des sirops

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

Autres usages

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

Connaissez-vous des cures recommandées (par exemple au printemps, en automne…)

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

Quelles plantes sauvages cueillez-vous vous-même ?

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

Quelles plantes sauvages utilisez-vous pour faire des cures (drainage du foie, renforcement des défenses immunitaires…) ?

Plante	Partie utilisée	Préparation	Effet thérapeutique éventuel

Connaissez-vous d'autres personnes que je pourrais contacter ? _____

Appendix B. Questionnaire Mayor.

Summary of the questionnaire

Formal information: name, sex, date of birth, address, place of birth, phone number, job, source of knowledge

For each plant, can you mention the origin, period of collect, medicinal and food uses, the mode of utilization, used parts (leaves, flowers, or roots), and mode of conservation (fresh, dried)?

Do you know other people I can contact?

Questionnaire

Enquêteur/Enquêtrice :

Nom/Prénom : _____

Adresse : _____

Tél : _____

Organisation : _____

Date de l'enquête : _____

Informateur/Informatrice :

Nom/Prénom : _____

Date de naissance : _____

Origine : _____

Sexe : _____

Adresse : _____

Tél : _____

Activité : _____

Etat civil : _____

Généralités :

Mode d'apprentissage du savoir :

- Familial :
- Etranger, voisin :
- Livre/média

Le savoir a-t-il été transmis (enfants, famille, apprentis, connaissances) :

Composition du savoir :

- Reconnaissance des plantes :
- Cultive certaines plantes :
- Conservation des plantes :

La personne connaît-elle (connaissait) d'autres personnes utilisant les plantes :

Nom(s)/Prénoms :

Adresse(s) :

Autres :

La plante:

Nom donné par l'informateur:

Nom scientifique :

Autres noms :

Famille :

Référence à l'herbier :

Référence photo :

Provenance :

- Sauvage ___ Cultivée___
- Localité :
- Station :

Cueillette :

- Epoque :
- Moment de la journée :
- Mode :
- Rituel :

Usages :	___ Thérapeutique	___ Alimentaire	___ Vétérinaire
	___ Technologique	___ Ludique	___ Symbolique
Parties utilisées :	___ Toute la plante	___ Racines	___ Tiges
	___ Feuilles	___ Fleurs	___ Fruits
Etat de la plante lors de l'utilisation :		___ Frais	___ Sec

Mode(s) d'utilisation(s) :

Mode(s) et durée de conservation :

Traditions, histoire, légende liées à la plante :

3.2. A comprehensive metabolite profiling of *Phyteuma orbiculare* L.

Salads, which are well regarded among the popuation, have more potential than plants used for making liqueurs whose market is continuously decreasing. A crucial point for selection is the absence of detailed analytical investigations already performed on the plants. A panel of around 30 candidates was collected from the wild and extracted successively with dichloromethane and methanol. Extracts were afterwards submitted to HPLC-MS-DAD and TLC dereplication to obtain finally only a small number of candidates for in depth phytochemical study.

Figure 4. *Phyteuma orbiculare* L.

The ethnobotanical survey on traditional food plants in Valais provided some information on the edible use of round-head rampion (*Phyteuma orbiculare* L. (Campanulaceae), Figure 4). The plant flowers have been traditionally eaten by shepherds as sweeties, whereas leaf rosettes have been consumed as salads. The round-head rampion is a wide spread perennial plants and grows in alpine and subalpine meadows. As it is large enough, the plant may be a suitable candidate for future cultivation. No phytochemical data was available neither on the species nor on the entire genus.

Dichloromethane and methanol extracts were subjected to a comprehensive metabolite profiling using a dereplication platform combining HPLC-PDA-MS and offline microprobe NMR analyses. Fatty acids, triterpenoids, and phenolic glycosides were identified online or after targeted isolation. The compounds include a new dimeric phenylpropanoid glucoside (tangshenoside VII) and two new triterpene saponins with unprecedented skeletons (phyteumosides A and B). Isolation and structure elucidation of the two saponins have been reported in the first contribution. Their structures were elucidated by spectroscopic and chemical methods, and were corroborated by X-ray diffraction analyses of the aglycons obtained after enzymatic hydrolysis.

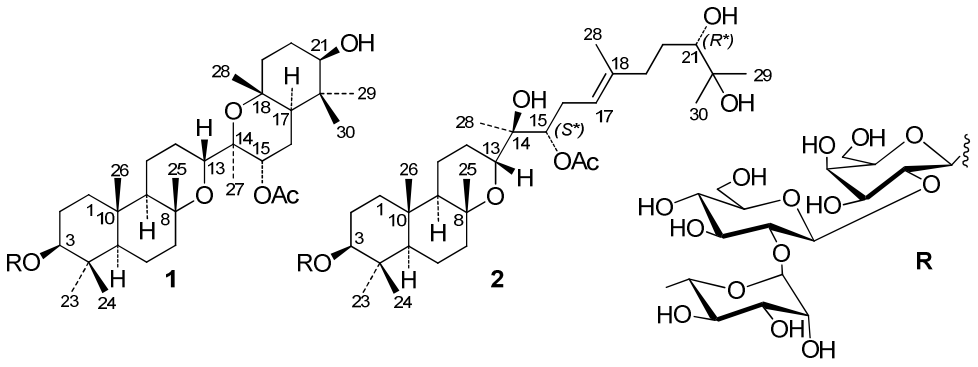

Figure 5. Phyteumosides A (**1**) and B (**2**), two new saponins with unique triterpenoid aglycons

The aglycon of **1** can be considered as an incompletely cyclized onoceroid or gammaceroid triterpene with two additional tetrahydropyran rings arising from oxygen bridges. Compound **2**, possesses a new 17-polypodene aglycon. Biosynthetically, both aglycons seem to derive from an unrearranged squalene molecule, which underwent incomplete cyclization.

From the aerial parts of *P. orbiculare*, a new dimeric phenylpropanoid glucoside (tangshenoside VII, Figure 6) was also isolated. Phenylpropanoid derivatives containing a HMG moiety were previously reported from systematically close genera and appear to be quite characteristic for plants of the family Campanulaceae.

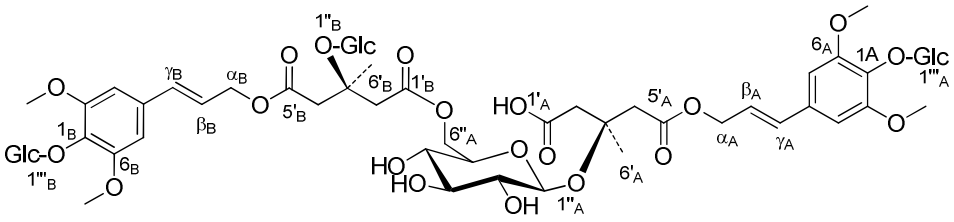

Figure 6. Tangshenoside VII, a new dimeric phenylpropanoid glucoside.

Quantitative data of compounds relevant for nutrition in edible parts were obtained according to standard procedures described in the literature. The leaves of *P. orbiculare* contained large amounts of β-carotene, potassium, magnesium, and calcium. The leaves possessed about 2.5 times more omega-3 than omega-6 fatty acids, which represents a nutritionally favorable ratio. Interestingly, the ratio was reversed in the flowers (10:7).

The fresh rampion is eaten as sweeties or as salad. Studies were consequently performed on fresh material (freeze dried leaves and flowers).

Finally, the study was extended to include the taxonomically closely related species *P. spicatum*, *P. hemisphaericum*, and *P. ovatum* (Figure 7). The HPLC-PDA-MS profiles showed a similar composition for *P. orbiculare, P. spicatum*, and *P. ovatum*, while free fatty acids and saponins were not detected in *P. hemisphaericum.* The data support the parallel use of these three species as food plants.

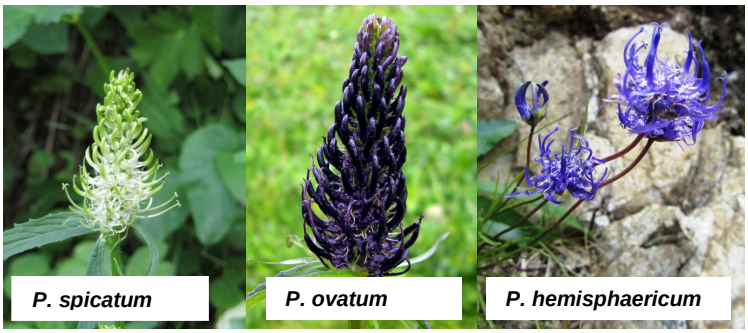

Figure 7. HPLC-PDA-MS revealed similarities in metabolite profiles of most species, but substantial differences were observed for *P. hemisphaericum.*

No known toxic compound neither toxic reported classes of molecules were discovered in *Phyteuma orbiculare*. Based on their chemical composition combined with pleasant gustatory properties, rampion species can be considered as a safe and healthy wild food plant and may be suitable candidates for future cultivation as food plants.

Collection of the plant material, structural elucidation of molecules based on spectroscopic and chemical analyses, crystallization of molecules, quantification of polyphenols, ORAC assay, preparation of the figures, and writing of the publication were my parts of the work. X-Ray analyses were performed by Dr. Neuburger and Dr. Wagner. Dr. Quitschau confirmed the assignments of the sugar moiety of phyteumosides A and B. Dr. Slacanin quantified vitamins, minerals, and fatty acids.

Christian Abbet

3.2.1 Phyteumosides A and B: new saponins with unique triterpenoid aglycons from Phyteuma orbiculare L.

Christian Abbet, Markus Neuburger, Trixie Wagner, Melanie Quitschau, Matthias Hamburger, Olivier Potterat.

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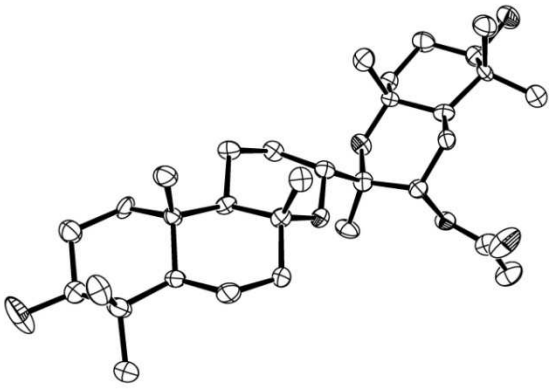

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Phyteumosides A and B: New Saponins with Unique Triterpenoid Aglycons from *Phyteuma orbiculare* L.

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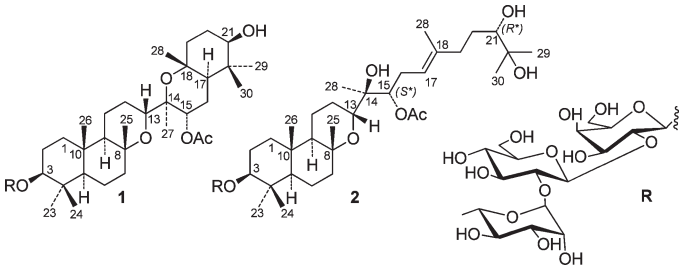
ABSTRACT



Phyteumosides A (1) and B (2), two saponins with unprecedented triterpenoid aglycons, were isolated from the aerial parts of *Phyteuma orbiculare* (Campanulaceae). Their structures were elucidated by spectroscopic and chemical methods and corroborated by X-ray diffraction analyses of the aglycons obtained through enzymatic hydrolysis. The aglycon of 1 can be considered as an incompletely cyclized onoceroid or gammaceroid triterpene with two additional tetrahydropyran rings arising from oxygen bridges. Compound 2 possesses a new 17-polypodene aglycon.

The round-headed rampion (*Phyteuma orbiculare* L., Campanulaceae) is a perennial herb which grows in subalpine and alpine regions of Central Europe. The leaves and the flowers were eaten in the past by the population of the Valais region (Switzerland) as a salad. In a study of forgotten traditional food plants, we investigated the aerial parts of *P. orbiculare*. No data have been reported on the secondary metabolites of this species nor the entire genus *Phyteuma*, but plants of the family Campanulaceae are known to contain triterpene saponins derived from oleanolic acid.[1] Here we report the isolation and structure elucidation of two new triterpene glycosides, phyteumosides A (**1**) and B (**2**), which possess unique triterpenic aglycons.

The aerial parts (226 g) of *P. orbiculare* were collected in June 2009 near Orsières in Valais, Switzerland. The dried plant material was defatted with CH_2Cl_2 and subsequently extracted with MeOH. Fractionation of the MeOH extract (43.7 g) by a combination of gel filtration on Sephadex LH-20 (MeOH) and flash chromatography on RP-18 (MeOH/H_2O gradient) afforded compounds **1** (51 mg) and **2** (40 mg). Both compounds gave purple spots on TLC after staining with vanillin/sulfuric acid.

The molecular formula of compound **1** ($[\alpha]^{20}_D$ −4.0 (*c* 0.27, MeOH)) was established as $C_{50}H_{84}O_{20}$ from the pseudomolecular $[M + H]^+$ ion at *m*/*z* 1005.5677 (calcd 1005.5629) in the HR-ESI-MS spectrum. ESI-MS^2 and MS^3 experiments in positive and negative modes gave fragment ions at *m*/*z* 857 ($[M - H - 146]^-$), 695

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Table 1. ^{1}H NMR and ^{13}C NMR Data of the Aglycon Portions of Phyteumosides A (**1**) and B (**2**)[a]

	phyteumoside A (**1**)				phyteumoside B (**2**)			
position	δ_H mult[b]	δ_C mult		HMBC	$\delta_H$mult[b]	δ_C mult		HMBC
1α	0.78	37.8	(t)		0.82	38.0	(t)	
1β	1.39				1.42			
2α	2.16, br m	27.0	(t)		2.16, br m	26.9	(t)	
2β	1.78				1.67			
3	3.28, dd (4.2, 11.5)	90.0	(d)	C-1′ C-2 C-4 C-23 C-24	3.35, dd (4.2, 11.8)	90.0	(d)	C-1′ C-2 C-4 C-23 C-24
4		40.0	(s)			40.0	(s)	
5	0.82	56.1	(d)		0.86	56.2	(d)	
6α	1.25	20.0	(t)		1.27	20.0	(t)	
6β	1.57				1.60			
7α	1.43	42.5	(t)		1.44	42.6	(t)	
7β	1.84				1.75			
8		75.5	(s)			75.3	(s)	
9	1.08	58.3	(d)	C-7 C-8 C-12	1.10, br d (12.2)	58.2	(d)	C-7 C-8 C-12
10		36.6	(s)			36.6	(s)	
11α	1.53	19.1	(t)		1.53	19.0	(t)	
11β	1.28				1.28			
12α	1.47	27.6	(t)		1.80	26.9	(t)	
12β	2.04				2.18			
13	3.83, dd (2.0, 11.3)	73.7	(d)		3.94, dd (1.9, 11.5)	73.1	(d)	
14		77.8	(s)			75.0	(s)	
15	5.75, dd (3.5, 4.6)	71.5	(d)	C-17 Ac(CO)	4.00	76.7	(d)	C-17 Ac(CO)
16α	1.87	23.1	(t)		1.82	27.5	(t)	
16β	2.05				2.06			
17	1.91	45.9	(d)	C-18 C-15	5.59	121.9	(d)	C-18 C-15
18		75.4	(s)			138.3	(s)	
19α	1.67	41.3	(t)		2.33, ddd (7.0, 9.6, 14.6)	38.0	(t)	
19β	1.83				2.62, ddd (4.6, 9.6, 14.2)			
20α	1.92	30.2	(t)		2.81	28.7	(t)	
20β	1.75				2.81			
21	3.54, dd (1.7, 10.4)	78.0	(d)	C-29 C-30	3.74, dd (1.4, 11.5)	78.8	(d)	C-29 C-30
22		38.9	(s)			72.9	(s)	
23	1.25, s	27.0	(q)	C-3 C-4 C-5	1.31, s	27.0	(q)	C-3 C-4 C-5
24	0.99, s	16.8	(q)	C-3 C-4 C-5	1.00, s	16.9	(q)	C-3 C-4 C-5
25	1.22, s	21.1	(q)	C-7 C-8 C-9	1.24, s	21.5	(q)	C-7 C-8 C-9
26	0.64, s	16.1	(q)	C-5 C-9 C-10	0.63, s	16.1	(q)	C-5 C-9 C-10
27	1.34, s	19.9	(q)	C-13 C-14	1.46, s	19.8	(q)	C-13 C-14
28	1.38, s	24.2	(q)	C-17 C-18 C-19	1.45, s	16.8	(q)	C-17 C-18 C-19
29	1.15, s	28.2	(q)	C-17 C-21 C-22 C-29	1.48, s	25.9	(q)	C-21 C-22 C-29
30	0.95, s	15.8	(q)	C-17 C-21 C-22 C-30	1.51, s	26.6	(q)	C-21 C-22 C-30
Ac(CO)		170.4	(s)			170.9	(s)	
Ac(Me)	2.09, s	21.6	(q)	Ac(CO)	2.03, s	21.4	(q)	Ac(CO)

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz), in pyridine-d_5 (δ in ppm, J in Hz). [b] Multiplicities of overlapped signals are omitted.

([M − H − 146 − 162]$^-$), and 653 ([M − H − 146 − 162 − 42]$^-$) and m/z 535 ([M + H − 146 − 162 − 162]$^+$), suggesting the presence of one acetyl group, one terminal rhamnose, and two hexose moieties. Acid hydrolysis of **1** (1 mg) with 2 N TFA (100 °C, 2 h) afforded L-rhamnose, D-galactose, and D-glucose, which were identified by GC−MS analysis after derivatization with L-cysteine methyl ester and silylation.[2]

The ^{13}C NMR spectrum of **1** exhibited 50 resonances including 10 methyl, 11 methylene, 22 methine, and 7 quaternary carbons. Among the signals assigned to the aglycon, four methine and three quaternary carbons were oxygenated (Table 1). The multiplicities together with the nine degrees of unsaturation suggested the presence of five rings in the aglycon portion, from which two were oxygen heterocycles. The ^{1}H NMR spectrum displayed eight tertiary methyl groups at δ_H 1.38, 1.34, 1.25, 1.22, 1.15, 0.99, 0.95, 0.64 ppm and confirmed the presence of an acetyl group (δ_H 2.09 ppm) (Table 1).

The carbon backbone and the substitution pattern of the aglycon were deduced from HSQC, HMBC (Table 1), and HSQC-TOCSY NMR data (Supporting Information). However, since no HMBC correlation was detected from H-13 to C-8, the exact connectivity of the epoxy bridges (8,13:14,18 or 8,14:13,18) could not be assigned. The NMR data were compatible with two pentacyclic scaffolds containing two tetrahydropyran or oxepan rings, respectively.

This ambiguity was finally resolved by an X-ray diffraction analysis of the aglycon (Figure 1). Treatment of

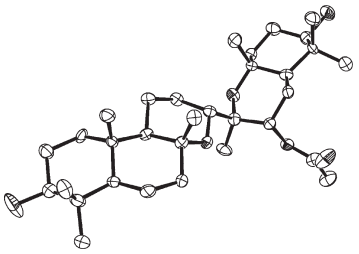

Figure 1. ORTEP drawing of aglycon **1a** with 50% probability displacement ellipsoids.

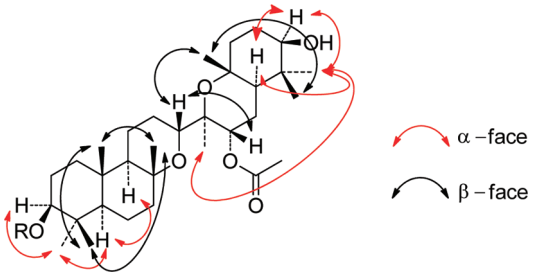

Figure 2. ROESY Correlations of phyteumoside A (**1**).

1 (20.0 mg) with a mixture of β-D-glucuronidase, hesperidinase, and β-galactosidase in acetate buffer (pH = 4.4) at 38 °C for 72 h yielded 7.0 mg of aglycon **1a** (ESI-MS: *m*/*z* 533.3 $[M + H]^+$; colorless needles from acetone/water).

The analysis also definitively confirmed the relative configuration which had been inferred from the 2D-ROESY correlations (Figure 2). Thus, the structure of **1a** is (3*S**,8*R**,13*R**,14*R**,15*S**,17*R**,18*R**,21*R**) 15-*O*-acetyl-8,13;14,18-diepoxy-17,18-diepi-13,18-seco-onocerane-3,15,21-triol.

With respect to the glycosidic portion, the ^{1}H NMR spectrum revealed the presence of three anomeric protons at δ_H 4.80, 5.57, and 6.29 ppm assigned to galactose, glucose, and rhamnose, respectively (Table 2). Vicinal coupling constants, *J*(1,2), of the anomeric H-atoms (δ_H 5.57 and 4.80 ppm (*J* = 7.6 Hz)) indicated a diaxial coupling and β-configuration for the galactose and glucose residues. The α-configuration of rhamnose was established on the basis of the ^{13}C NMR chemical shifts of C-3 and C-5.[3] The identity of the oligosaccharide chain was established by ^{1}H−^{1}H-COSY, HSQC, HMBC, HSQC-TOCSY, 1D TOCSY, and ROESY experiments. Starting from the anomeric protons of each sugar and from the methyl group of the rhamnose, all protons and carbons could be assigned within each spin system. In particular, correlations of H-1/H-2 and H-2/H-3 in the COSY spectrum along with the small coupling constant for H-3/H-4 (*J* = 2.9 Hz) supported the assignment of β-galactopyranose protons, while the signals δ_H 5.57, 4.20, 4.14, 3.98, and 3.62 ppm showed the typical spin system of a β-glucopyranosyl unit.

Table 2. ^{1}H and ^{13}C NMR Data of the Glycosidic Portion of **1**[a,b]

	position	δ_H[c] mult	δ_C
Gal	1	4.80, d (7.6)	106.0
	2	4.65, dd (7.7, 9.3)	77.8
	3	4.38, dd (2.9, 9.4)	76.7
	4	4.31, br s	70.8
	5	3.96	76.8
	6	4.34, dd (4.0, 8.8)	62.8
		4.34, dd (4.0, 8.8)	
Glc	1	5.57, d (7.6)	102.4
	2	4.20, dd (7.6, 9.0)	79.8
	3	4.14, dd (8.8, 9.0)	78.5
	4	3.98, dd (8.6, 9.2)	73.1
	5	3.62, ddd (5.5, 9.6, 9.2)	77.4
	6	4.09	63.6
		4.26, dd (9.6, 12.0)	
Rha	1	6.29, br s	102.3
	2	4.67	73.1
	3	4.69	73.0
	4	4.26	74.7
	5	4.93, dq (6.2, 9.2)	69.9
	6	1.73, d (6.2)	19.2

[a] ^{1}H NMR(500 MHz) and ^{13}C NMR (125 MHz), in pyridine-d_5 (δ in ppm, *J* in Hz). [b] Data of **2** showed deviations of < 0.01 (^{1}H) or < 0.04 (^{13}C) ppm and are provided in the Supporting Information. [c] Multiplicities of overlapped signals are omitted.

HMBC correlations (^3J) observed between H-1 of glucose (δ_H 5.57 ppm) and C-2 of galactose (δ_C 77.8 ppm) and between H-1 of rhamnose (δ_H 6.29 ppm) and C-2 of glucose (δ_C 79.8 ppm) enabled the sugar chain to be assigned as [α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl]. ROESY correlations further supported the interglycosidic linkages. The sugar chain was linked to C-3 of the aglycon based on the correlation between H-1 of galactose (δ_H 4.80 ppm) and C-3 (δ_C 90.0 ppm). This trisaccharidic moiety has not been yet reported.

Phyteumoside B (**2**) ($[\alpha]^{20}_D$ −28.9 (*c* 0.11, MeOH)) was assigned the molecular formula $C_{50}H_{86}O_{21}$ from the pseudomolecular $[M + H]^+$ ion at *m*/*z* 1023.5790 (calcd 1023.5734) in the HR-ESI-MS spectrum. The presence of D-galactose, D-glucose and L-rhamnose was established by acid hydrolysis and GC analysis. The ^{1}H and ^{13}C NMR signals assigned to the glycosidic portion were identical to those observed in **1** revealing the same oligosaccharide chain. The position of the glycosidic chain was inferred from the HMBC correlation between H-1 of the galactose and C-3 of the aglycon.

With regard to the aglycon portion, the multiplicities together with the eight degrees of unsaturation suggested the presence of three rings and a double bond. While the NMR signals including 2D correlations (Table 1, Figure 3) of rings A and B and the tetrahydropyran ring fused to ring B remained practically unchanged compared to **1**, significant differences were observed for the rest of the molecule. The methyls Me-29 and Me-30 were attached to an oxygenated carbon at δ_C 72.9 ppm and presented no HMBC correlation with C-17 in contrast to compound **1** which confirmed

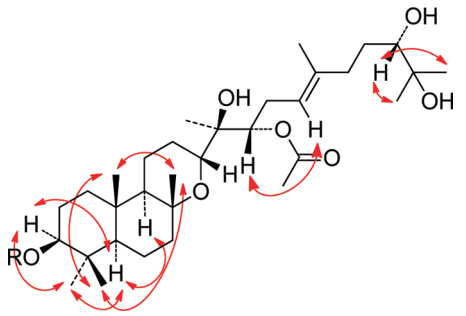

Figure 3. ROESY Correlations of phyteumoside B (**2**).

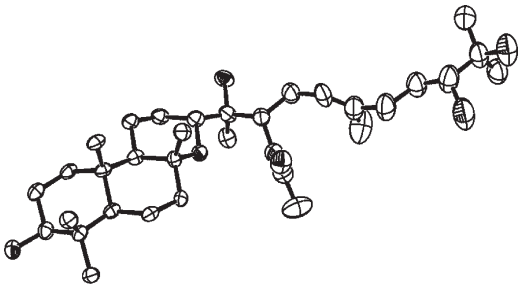

Figure 4. ORTEP drawing of aglycon **2a** with 50% probability displacement ellipsoids.

the absence of cyclization between C-17 and C-22. The double bond could be assigned to C-17(18) from the C-15 to C-17 spin system detected in the HSQC-TOCSY spectrum and the HMBC correlations from Me-28 to C-17, C-18 and C-19. This implied the absence of an oxygen bridge between C-14 and C-18. The position of the acetyl group was confirmed from the HMBC correlation between H-15 and the acetyl CO.

The complete structure of the aglycon of **2** including its relative configuration was also established by X-ray diffraction analysis (Figure 4).

Incubation of **2** (15 mg) with a mixture of glycosidases under the same condition as for **1** and subsequent purification by semipreparative HPLC afforded 1.5 mg of aglycon **2a** (ESI-MS: *m*/*z* 551.2 $[M + H]^+$, colorless needles from $MeCN/H_2O$).

The structure of **2a** was established as (3*S**,8*R**,13*R**, 14*R**,15*S**,21*R**) 15-*O*-acetyl-8,13-epoxy-17-polypoden-3,15,21,22-pentol. It is noteworthy that the relative configuration of **1a** and **2a** is identical.

The structure of **1** can be regarded as derived from a new secoonoceroid (or bissecogammaceroid) skeleton, but the reversed configuration at C-17 and C-18 is, to our knowledge, unique in these groups of triterpenes. The existence of two tetrahydropyran rings in the center of the cyclized carbon chain is also unprecedented. Interestingly, gammacerane triterpenes have been mainly reported from sediments,[4] bacteria,[5] and ferns.[6] In higher plants, few gammacerane triterpenoids have been reported in taxonomically scattered species including *Abies* species (Pinaceae),[7] *Ailanthus grandis* (Simaroubaceae),[8] and *Coriandrum sativum* (Apiaceae).[9] Onoceroids are rare in nature and mostly found in club mosses and ferns.[10] One representative, α-onocerin, has been, however, reported in various higher plants, in particular *Ononis* species (Fabaceae).[11] There are only few natural products with structurally analogous features as in **1** and **2**. Labdane diterpenes such as microtropiosides A-F[12] and tarapacol[13] possess the same tricylic system as **1** and **2**. Compound **1** exhibits also some similarities with colysanoxide, an onoceroid triterpene from fern species of the genus *Colys*, possessing a tetrahydropyrane ring fused to the ring E as in **1**.[14] However, the relative configuration at C-17 and C-18 in colysanoxide is opposite to **1**.

Biosynthetically, both aglycons seem to derive from an unrearranged squalene molecule, which underwent incomplete cyclization. The presence of OH groups at both C-3 and C-21 in **1** would agree with cyclization of a squalene bisepoxide from both ends as described for the onocerane skeleton.[15] Interestingly, triterpenes with the same tricyclic system as in **2** have been obtained by incubation of squalene diols with a squalene cyclase from *Alicyclobacillus acidcaldarius*.[16]

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Supporting Information Available. Experimental procedures, MS and NMR spectra of compounds **1** and **2**, X-ray crystallographic data (CIF), and NMR data of aglycons **1a** and **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information

Phyteumosides A and B: New saponins with unique triterpenoid aglycons from *Phyteuma orbiculare* L.

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S1. Experimental Section.

General Experimental Procedures. Sephadex LH-20 was purchased from *GE Healthcare*. HP-diaion was obtained from *Sigma-Aldrich*. Flash chromatography was performed on a Sepacore® chromatography system (*Büchi Labortechnik*) equipped with a pre-packed RP-18 cartridge (40 x 150 mm, 40-63 μm, *Büchi*). Optical rotation was measured on a JASCO P-2000 automatic digital polarimeter. NMR spectra were recorded on a 500 MHz Avance III spectrometer (*Bruker*) equipped with a 5 mm BBI probe (^{13}C-NMR) or a 1 mm TXI microprobe (^{1}H- and 2D-NMR); at 500 (^{1}H) and 125 MHz (^{13}C), δ in ppm rel. to Me_4Si, *J* in Hz. Standard pulse sequences of the software Topsin 2.1 were used. ESI-MS spectra were obtained on a *Esquire 3000 plus* ion trap mass spectrometer (*Bruker*). HR-ESI-MS spectra were measured on a LTQ Orbitrap XL mass spectrometer (*Thermo Scientific*). Sugar analysis was performed on a *HP 5890 Series II* gas chromatograph equipped with a *HP 5971* mass selective detector (*Hewlett Packard*); injector temp. 180°C; detector temp. 260°C; He as carrier gas. β-D-glucuronidase, hesperidinase, and β-galactosidase were from Sigma. TLC was conducted on precoated silica gel plates GF_{254} (*Merck*).

Plant Material. The aerial parts of *Phyteuma orbiculare* L. (round-headed rampion) were collected by C. Abbet on 24th June 2009 in L'Amônaz, near Orsières, Valais, Switzerland. The plant was identified by C. Rey, Senior Scientist at the Agroscope of Changins, Châteauneuf, Switzerland. A voucher specimen (Nr 532) is preserved at the Division of Pharmaceutical Biology, University of Basel, Switzerland.

Extraction and Isolation. The dried powdered aerial parts (226 g) of *P. orbiculare* were extracted at r.t. with CH_2Cl_2 (3 x 2000 mL, each 24 h) followed by MeOH (3 x 2000 mL, each 24 h). After evaporation to dryness under reduced pressure, a portion (42.3 g) of the MeOH extract (43.7 g) was dissolved in 100 mL water and then loaded onto a Diaion HP-20 column (70 x 400 mm i.d.) eluted with water (7 L), followed by MeOH (15 L). A portion (5.0 g) of the MeOH fraction (6.4 g) was separated on a Sephadex LH-20 column (7 x 100 cm i.d.) with MeOH to give 15 fractions (Fr. 1-15). Fr. 4 (539 mg) was submitted to flash chromatography on RP-18 with MeOH/H_2O (20 to 100%) as eluent. Final purification of the fraction eluted with 100% MeOH, (75 mg) by Sephadex LH-20 (MeOH) yielded **1** (51 mg). Separation of Fr. 3 (263 mg) by flash chromatography on RP-18 with MeOH/H_2O (20% to 100%) afforded **2** (40 mg).

Acid hydrolysis. Saponin **1** (0.7 mg) or saponin **2** (1 mg) were heated at 105°C for 2 h in 0.7 (**1**) or 1 ml (**2**) of 2M TFA. The solutions were extracted three times with 1 mL $CHCl_3$. TLC analysis of the organic phase revealed decomposition of the aglycone. The aq. phase was dried and the residue re-dissolved in anhydrous pyridine. The sugars were derivatized with L-cysteine methyl ester hydrochloride (200 μl, 60°C, 1 h) and subsequently silylated with hexamethyldisilzane and chlorotrimethylsilane (*Fluka*) in pyridine (2:1:10, 300 μl; 60°C, 30 min). GC Analysis on a capillary DB-225MS column (30 m x 0,25 mm i.d., 0,25 μm; *Agilent*; column temp. 150°C for 2 min, then 5°C/min. to 210°C, then 10°C/min to 240°C).

Enzymatic hydrolysis. Saponin **1** (20 mg) was incubated with β-D-glucuronidase (40 mg, 77'040 UI), hesperidinase (200 mg, 3.6 UI) and β-galactosidase (160 mg, 1376 UI) in acetate buffer (20 ml, pH 4.4) for 72 h at 38°C. Extraction with EtOAc (3 x 20 ml) provided 7 mg of the aglycon **1a**, which was finally recrystallized from H_2O/acetone (10:1) to give colorless needles.

Compound **2** (15 mg) was hydrolysed following the same procedure with β-D-glucuronidase (30 mg, 57'780 UI), hesperidinase (150 mg, 2.7 UI) and β-galactosidase (120 mg, 1032 UI) in acetate buffer (15 ml, pH 4.4), at 38°C for 72 h. Extraction with EtOAc (3 x 15 ml) provided 5 mg of crude **2a** which was purified by semi-preparative HPLC (*Agilent* series 1100 system; *Waters* SunFire^{TM} Prep C18 column (150 x 10 mm i.d., 5 μm), MeCN/H_2O (60% to 100%), 205 nm) to give pure **2a** (1.5 mg). Recrystallization from H_2O/MeCN (10:1) afforded colorless needles.

S2. Crystal Data of the aglycon of Phyteumoside A (**1a**).

Formula $C_{32}H_{56.76}O_{8.38}$, M = 571.77, F(000) = 1248, colorless plate, size 0.010 · 0.090 · 0.270 mm^3, orthorhombic, space group P 2_1 2 2_1, Z = 4, a = 6.4777(15) Å, b = 16.504(5) Å, c = 29.762(7) Å, α = 90°, β = 90°, γ = 90°, V = 3181.9(14) $Å^3$, $D_{calc.}$ = 1.193 Mg m^{-3}. The crystal was measured on a Bruker Kappa Apex2 diffractometer at 123K using graphite-monochromated Mo K_α-radiation with λ = 0.71073 Å, Θ_{max} = 27.098°. Minimal/maximal transmission 0.99/1.00, μ = 0.085 mm^{-1}. Apex2[1] has been used for integration. From a total of 10935 reflections, 3995 were independent (merging r = 0.122). From these, 3968 were considered as observed (I>2.0σ(I)) and were used to refine 373 parameters. The structure was solved by direct methods using the program Superflip[2]. Least-squares refinement against Fsqd was carried out on all non-hydrogen atoms using the program CRYSTALS[3]. R = 0.0880 (observed data), wR = 0.2294 (all data), GOF = 0.9538. Minimal/maximal residual electron density = -1.12/1.02 e $Å^{-3}$. Sheldrick weights[4] were used to complete the refinement. Plots were produced using ORTEP3 for Windows[5]. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center, the deposition number is 809711. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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S3. Crystal Data of the aglycon of Phyteumoside B (**2a**).

Formula $C_{32}H_{70}O_{14}$, M = 678.90, F(000) = 1456, colorless plate, size 0.010 · 0.150 · 0.250 mm^3, monoclinic, space group C 2, Z = 4, a = 50.084(4) Å, b = 5.9938(6) Å, c = 13.2247(10) Å, α = 90°, β = 105.051(4)°, γ = 90°, V = 3833.8(6) $Å^3$, $D_{calc.}$ = 1.18 Mg · m^{-3}. The crystal was measured on a Bruker SMART diffractometer at 173K using monochromated Cu K_α-radiation with λ = 1.54180 Å, Θ_{max} = 66.997°. Minimal/maximal transmission 0.93/0.99, μ = 0.749 mm^{-1}. SAINT[6] has been used for integration. From a total of 23579 reflections, 6224 were independent (merging r = 0.069). From these, 4707 were considered as observed (I>2.0σ(I)) and were used to refine 443 parameters. The structure was solved by direct methods using the program SHELXS 86 [4]. Least-squares refinement against F was carried out on all non-hydrogen atoms using the program CRYSTALS [3]. R = 0.0833 (observed data), wR = 0.1160 (all data), GOF = 0.9263. Minimal/maximal residual electron density = -0.70/0.70 e $Å^{-3}$. Chebychev polynomial weights[7] were used to complete the refinement. Plots were produced using ORTEP3 for Windows[8]. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center, the deposition number is 809712. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

(6) *Siemens Industrial Automation, SAINT: Area-Detector Integration Software.* Madison, Wisconsin, 1995.
(7) Watkin, D.J. *Acta Cryst* **1994**, *A50*, 411.
(8) Farrugia, L.J. *J Appl Crystallogr* **1997**, *30*, 565

Table 1. NMR Data of the Aglycons of Phyteumoside A (**1a**) and B (**2a**)[a].

	1a				**2a**			
position	$\delta_H$mult.[b]	δ_C mult.		HMBC	$\delta_H$mult.[b]	δ_C mult.		HMBC
1α	0.96	37.4	(t)		0.97	37.3	(t)	
1β	1.58				1.60			
2α	1.56	27.3	(t)		1.55	27.3	(t)	
2β	1.61				1.62			
3	3.17, m	79.0	(d)	C-2 C-4 C-23 C-24	3.19, dd (12.0, 4.0)	78.7	(d)	C-2 C-4 C-23 C-24
4		39.0	(s)			38.8	(s)	
5	0.88	55.3	(d)		0.89	55.3	(d)	
6α	1.32	19.7	(t)		1.35	19.5	(t)	
6β	1.64				1.67			
7α	1.32	41.8	(t)		1.72	41.8	(t)	
7β	1.68				1.36			
8		74.8	(s)			75.1	(s)	
9	1.05	57.7	(d)	C-7 C-8 C-12	1.07	57.6	(d)	C-7 C-8 C-12
10		36.6	(s)			36.5	(s)	
11α	1.29	18.6	(t)		1.32	18.2	(t)	
11β	1.58				1.59			
12α	1.29	26.9	(t)		1.33	26.5	(t)	
12β	1.84				1.71			
13	3.62, dd (2.1, 9.7)	73.4	(d)		3.48, dd (2.1, 11.1)	73.4	(d)	
14		77.6	(s)			73.8	(s)	
15	5.33, dd (3.9, 4.4)	70.9	(d)	C-17 Ac(CO)	4.96, dd (3.1, 9.8)	76.5	(d)	C-17 Ac(CO)
16α	1.62	22.2	(t)		2.30, m	28.2	(t)	
16β	1.82				2.45, m			
17	1.60	45.0	(d)	C-18 C-15	5.16, dd (7.3, 7.6)	121.3	(d)	C-18 C-15
18		74.8	(s)			136.7	(s)	
19α	1.44	40.2	(t)		2.08, m	36.6	(t)	
19β	1.66				2.15, m			
20α	1.44	29.1	(t)		1.36	29.1	(t)	
20β	1.73				1.57			
21	3.30, dd (1.7, 10.6)	78.5	(d)	C-29 C-30	3.28, dd (1.7, 10.4)	77.5	(d)	C-29 C-30
22		38.0	(s)			72.7	(s)	
23	0.94, s	28.2	(q)	C-3 C-4 C-5 C-22	0.95,s	28.1	(q)	C-3 C-4 C-5 C-22
24	0.73, s	15.4	(q)	C-3 C-4 C-5 C-21	0.74,s	15.2	(q)	C-3 C-4 C-5 C-21
25	1.18, s	20.7	(q)	C-7 C-8 C-9	1.20,s	20.2	(q)	C-7 C-8 C-9
26	0.71, s	15.8	(q)	C-5 C-9 C-10	0.71,s	15.7	(q)	C-5 C-9 C-10
27	0.99, s	19.2	(q)	C-13 C-14	1.11,s	19.9	(q)	C-13 C-14
28	1.21, s	23.6	(q)	C-17 C-18 C-19	1.60,s	16.0	(q)	C-17 C-18 C-19
29	0.70, s	14.9	(q)	C-17 C-21 C-22 C-29	1.11,s	23.4	(q)	C-21 C-22 C-29
30	0.89, s	27.4	(q)	C-17 C-21 C-22 C-30	1.16,s	26.2	(q)	C-21 C-22 C-30
Ac(CO)		169.9	(s)			169.9	(s)	
Ac(Me)	2.00, s	21.2	(q)	Ac(Me)	2.00,s	21.0	(q)	Ac(Me)

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in $CDCl_3$, (δ in ppm, J in Hz). [b]Multiplicities of overlapped signals are omitted.

Table 2. ^{1}H and ^{13}C NMR Data of the glycosidic Portion of **2**[a]

	position	δ_H, mult.[b]	δ_C
Gal	1	4.80, d (7.4)	105.9
	2	4.64, dd (7.7, 9.3)	77.7
	3	4.39, dd (2.9, 9.4)	76.7
	4	4.32, br s	70.8
	5	3.96	76.8
	6	4.33, dd (4.0, 8.8)	62.8
		4.34, dd (4.0, 8.8)	
Glc	1	5.57, d (7.6)	102.4
	2	4.20, dd (7.6, 9.0)	79.8
	3	4.14, dd (8.8, 9.0)	78.4
	4	3.98, dd (8.6, 9.2)	73.1
	5	3.61, ddd (5.5, 9.6, 9.2)	77.4
	6	4.10	63.6
		4.26, dd (9.6, 12.0)	
Rha	1	6.29, br s	102.3
	2	4.67	73.1
	3	4.69	73.0
	4	4.26	74.7
	5	4.94, dq (6.2, 9.2)	69.9
	6	1.73, d (6.2)	19.3

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in pyridine d_5, (δ in ppm, J in Hz). [b]Multiplicities of overlapped signals are omitted.

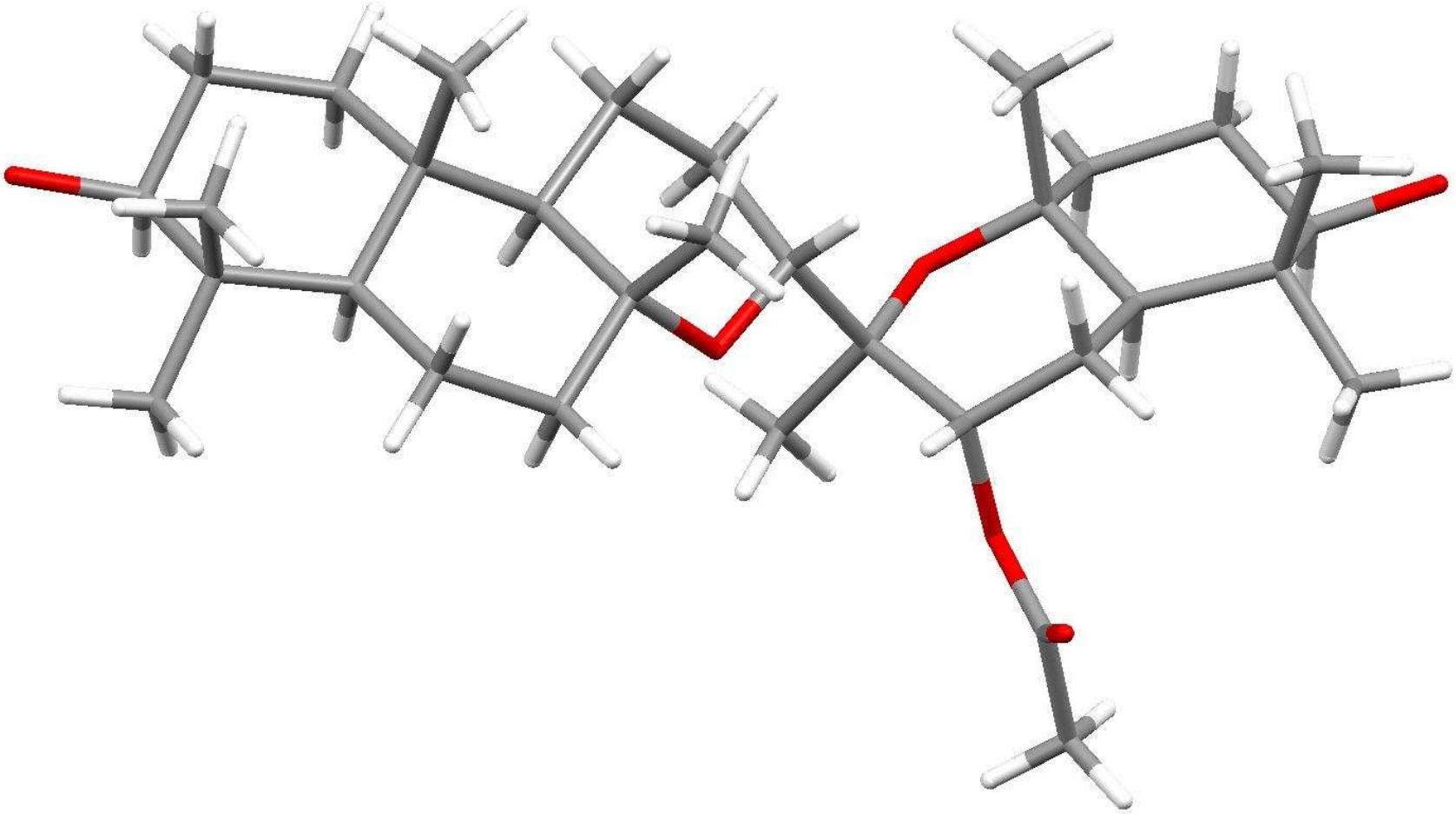

Figure S1. . X Ray Structure of Phyteumoside A (**1**) (Capped Stick Drawing).

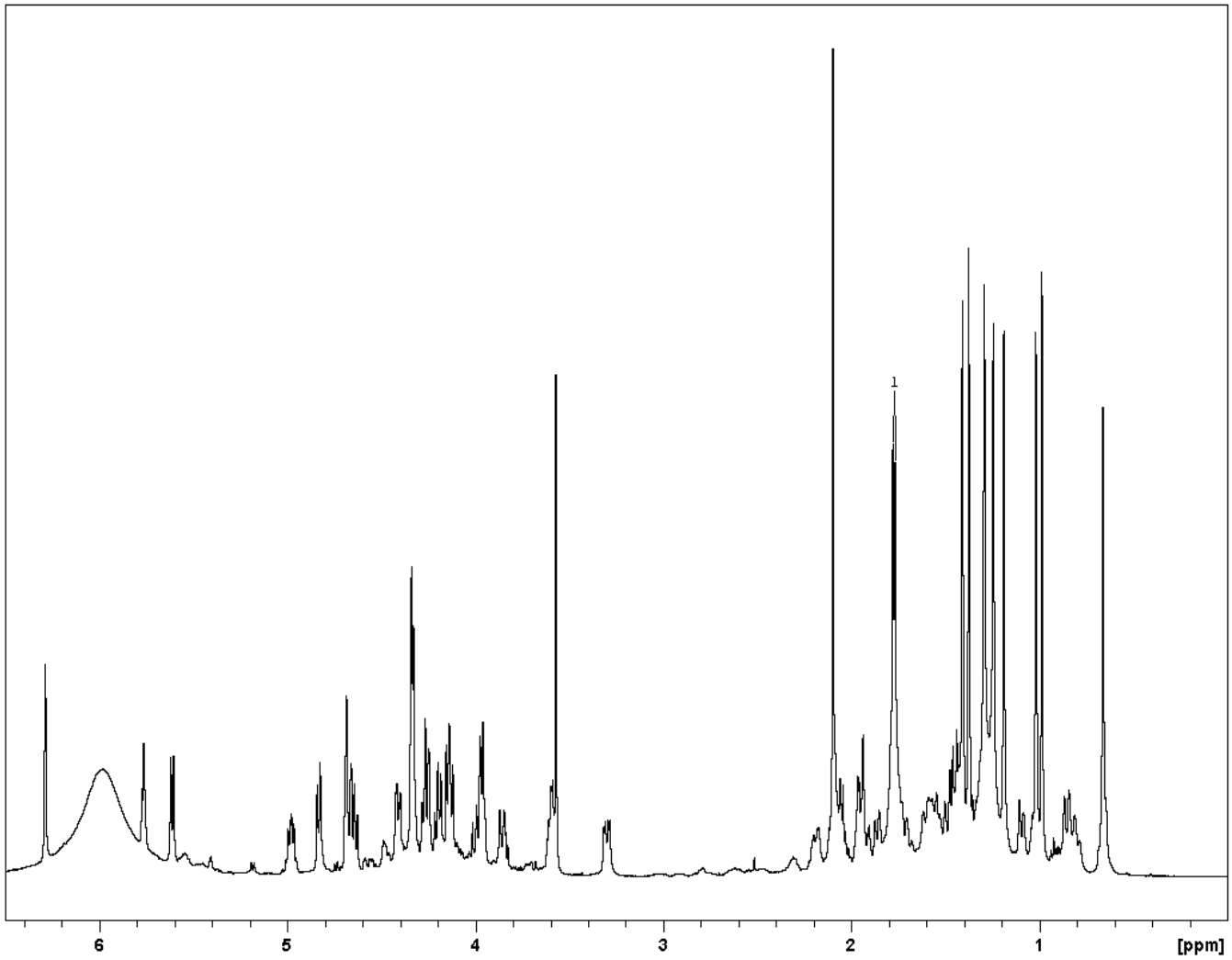

Figure S2. ^{1}H NMR Spectrum of Phyteumoside A (**1**).

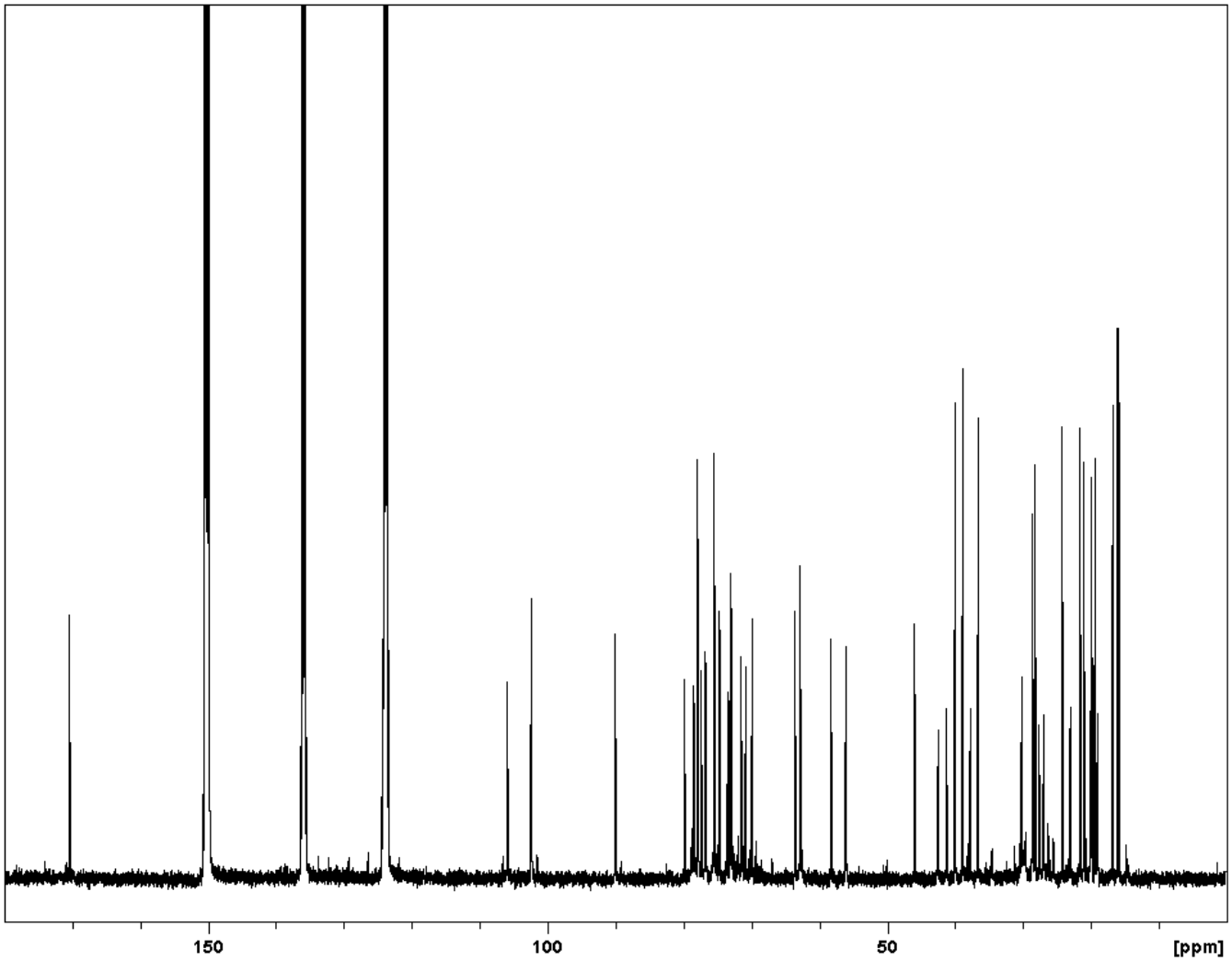

Figure S3. ^{13}C NMR Spectrum of Phyteumoside A (**1**).

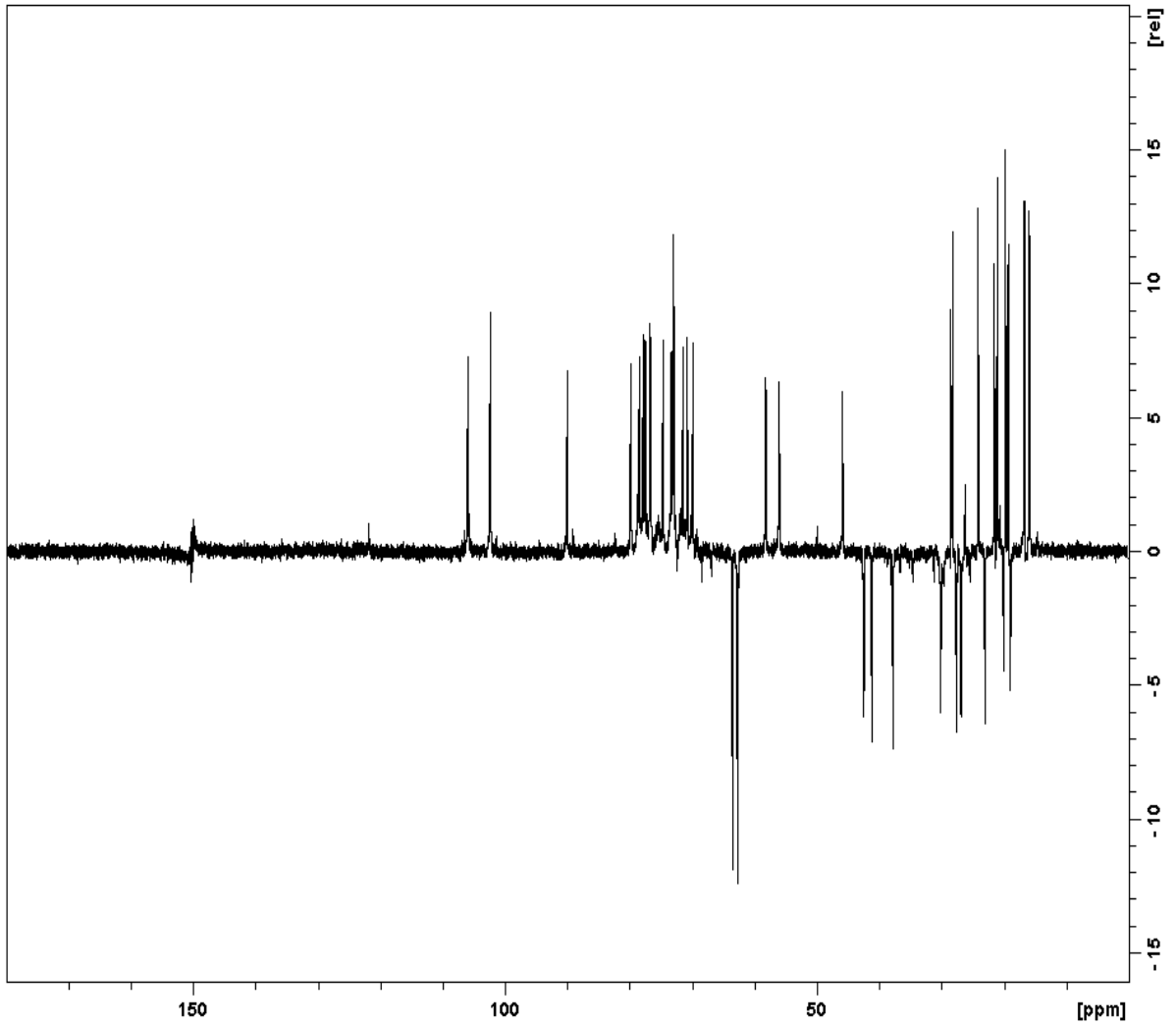

Figure S4. DEPT Spectrum of Phyteumoside A (**1**).

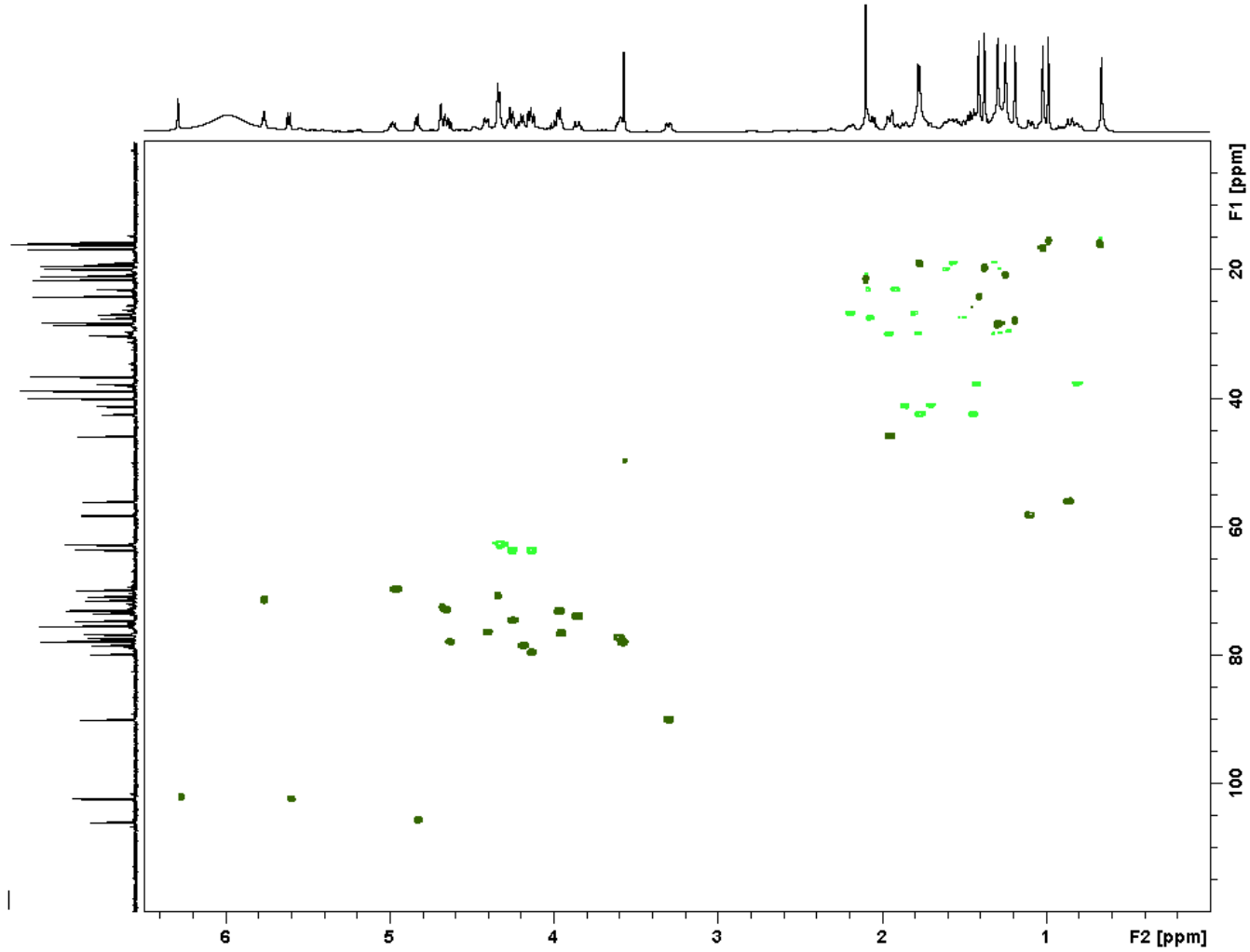

Figure S5. HSQC Spectrum of Phyteumoside A (**1**).

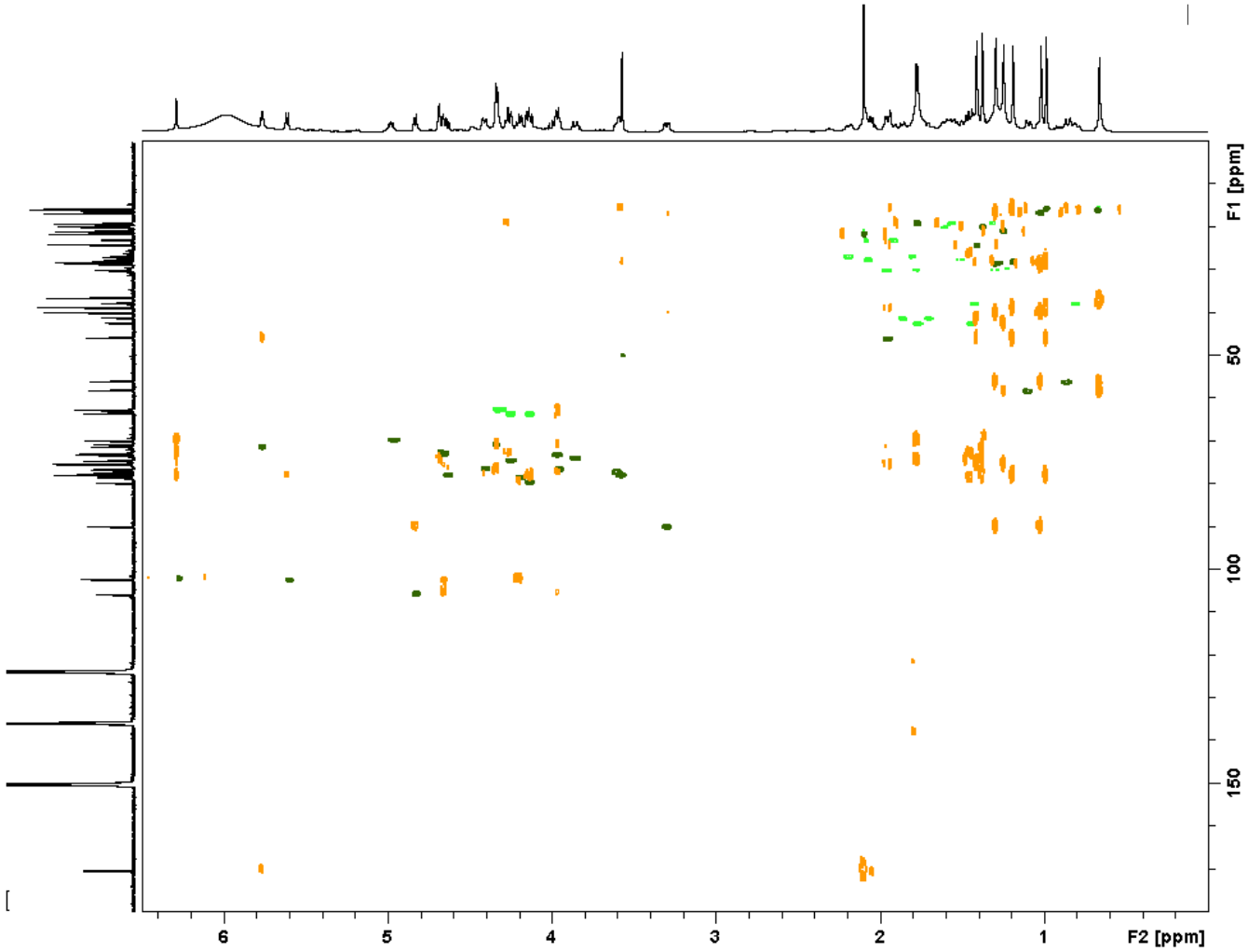

Figure S6. HMBC Spectrum of Phyteumoside A (**1**).

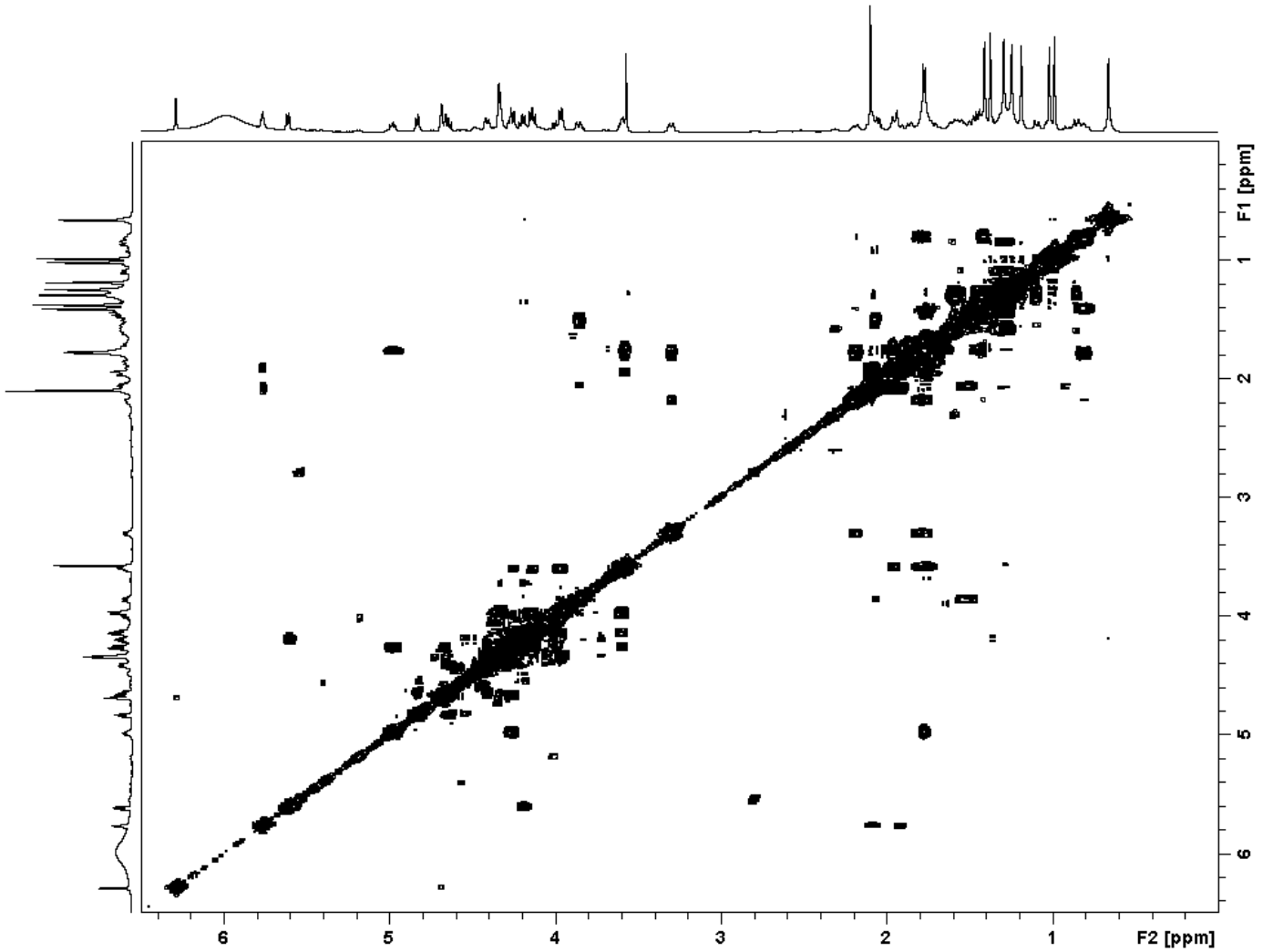

Figure S7. COSY Spectrum of Phyteumoside A (**1**).

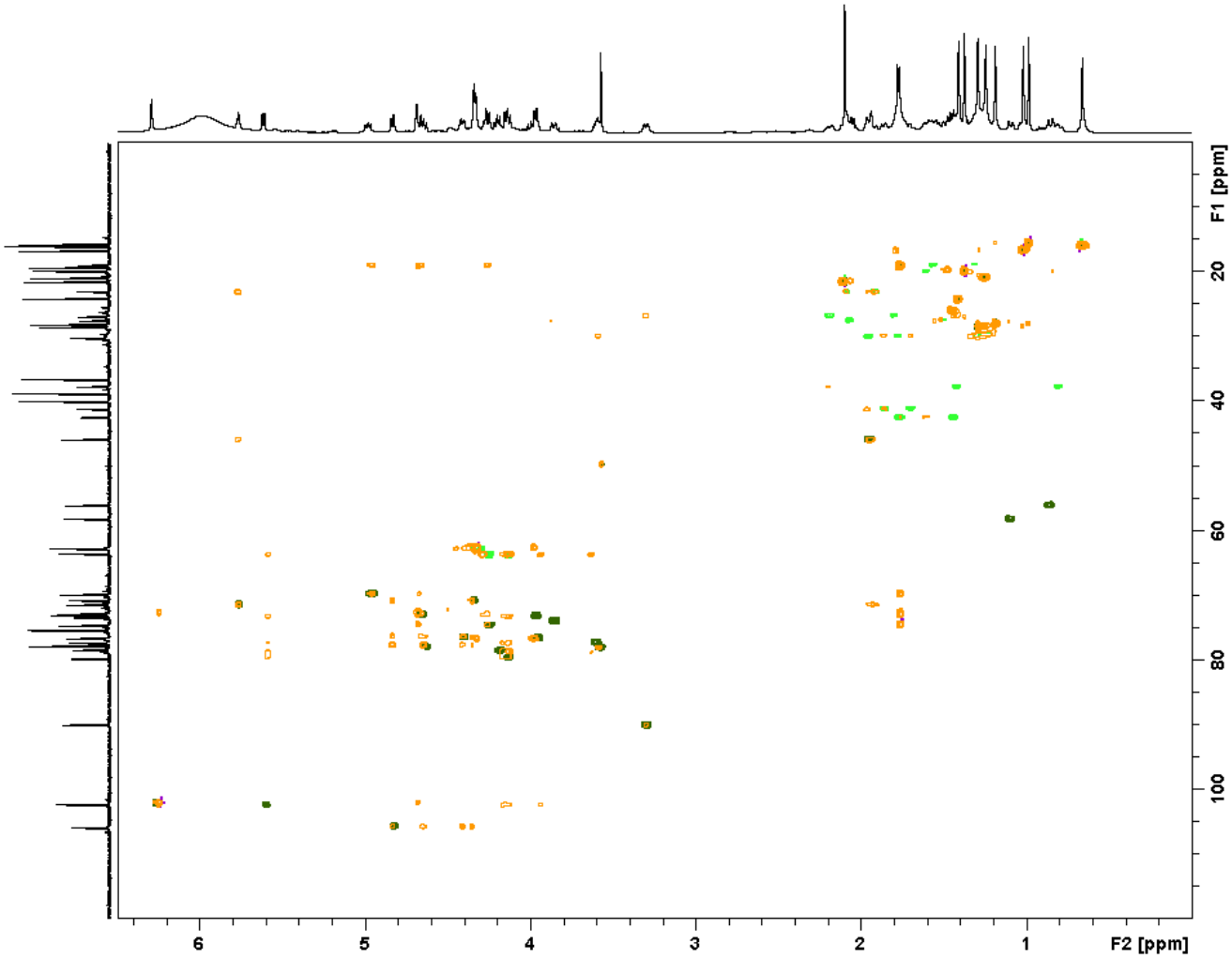

Figure S8. TOCSY Spectrum of Phyteumoside A (**1**).

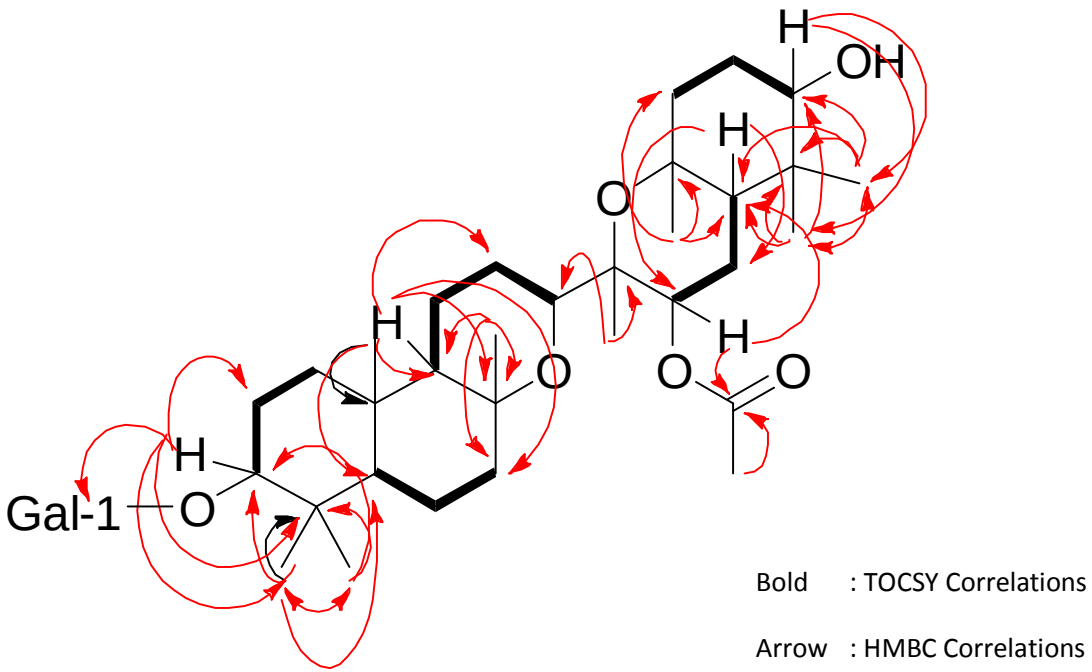

Figure S9. HMBC and TOCSY correlations of Phyteumoside A (**1**).

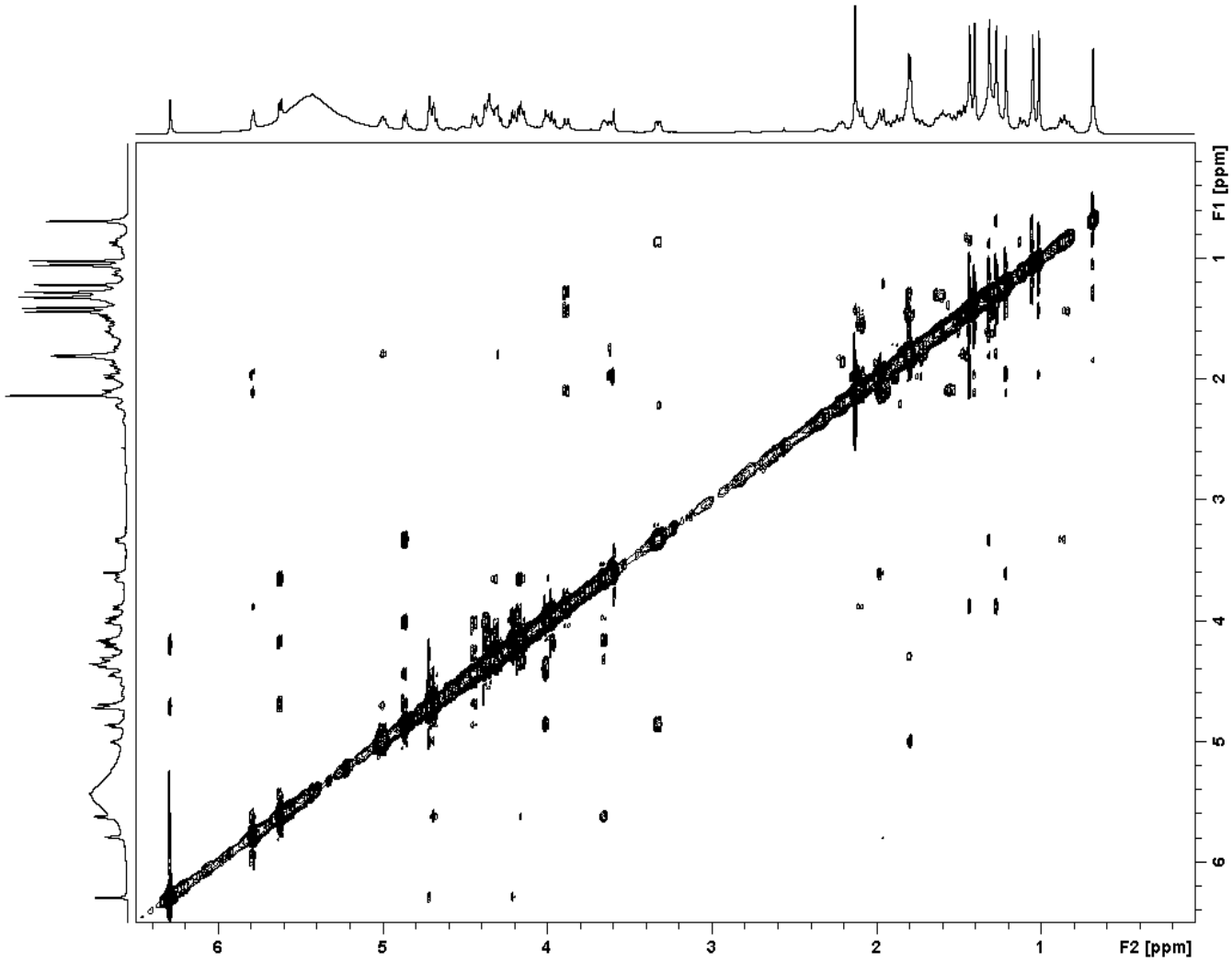

Figure S10. ROESY Spectrum of Phyteumoside A (**1**).

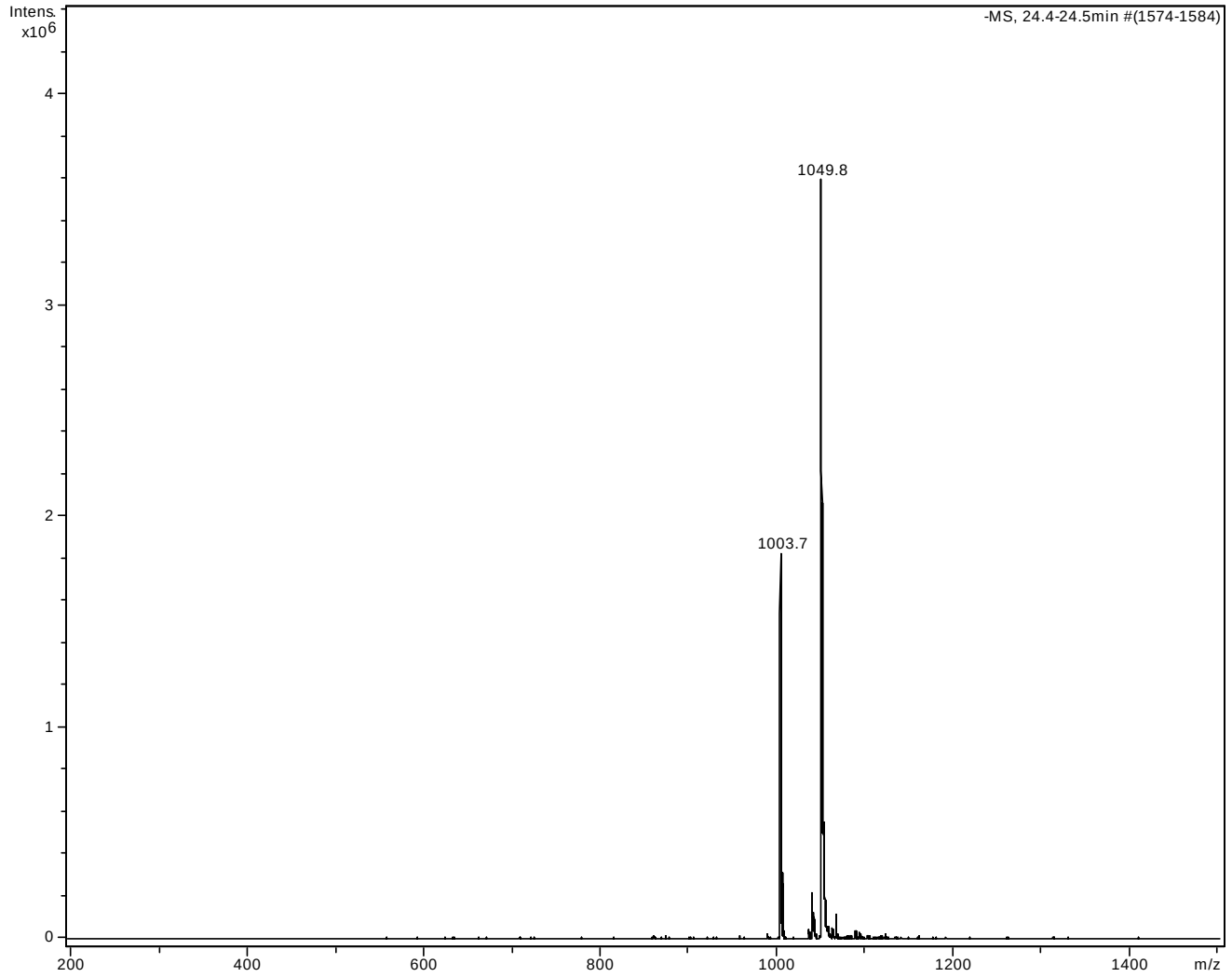

Figure S11. ESI-MS Spectrum of Phyteumoside A (**1**).

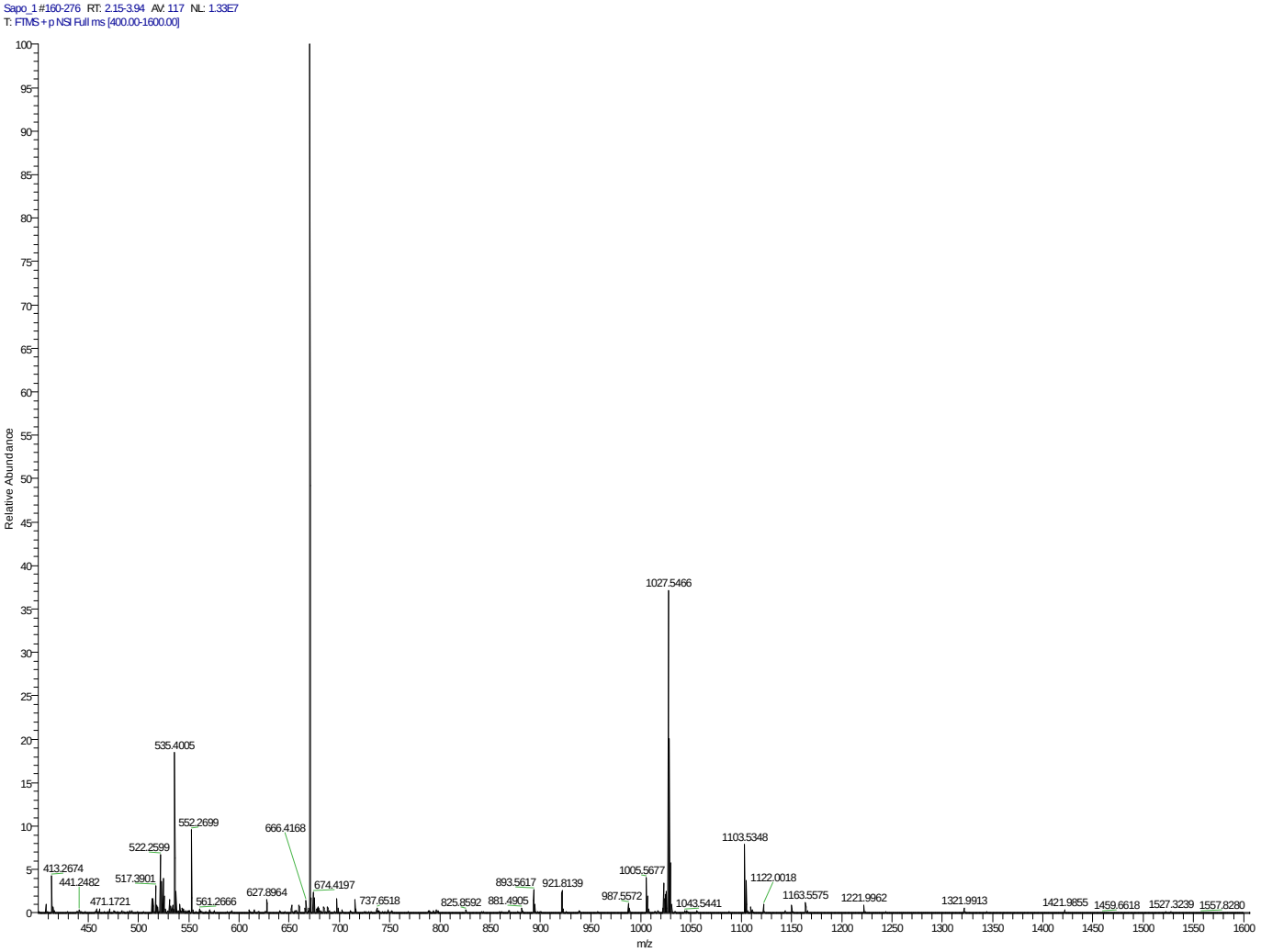

Figure S12. HR-ESI-MS Spectrum of Phyteumoside A (**1**).

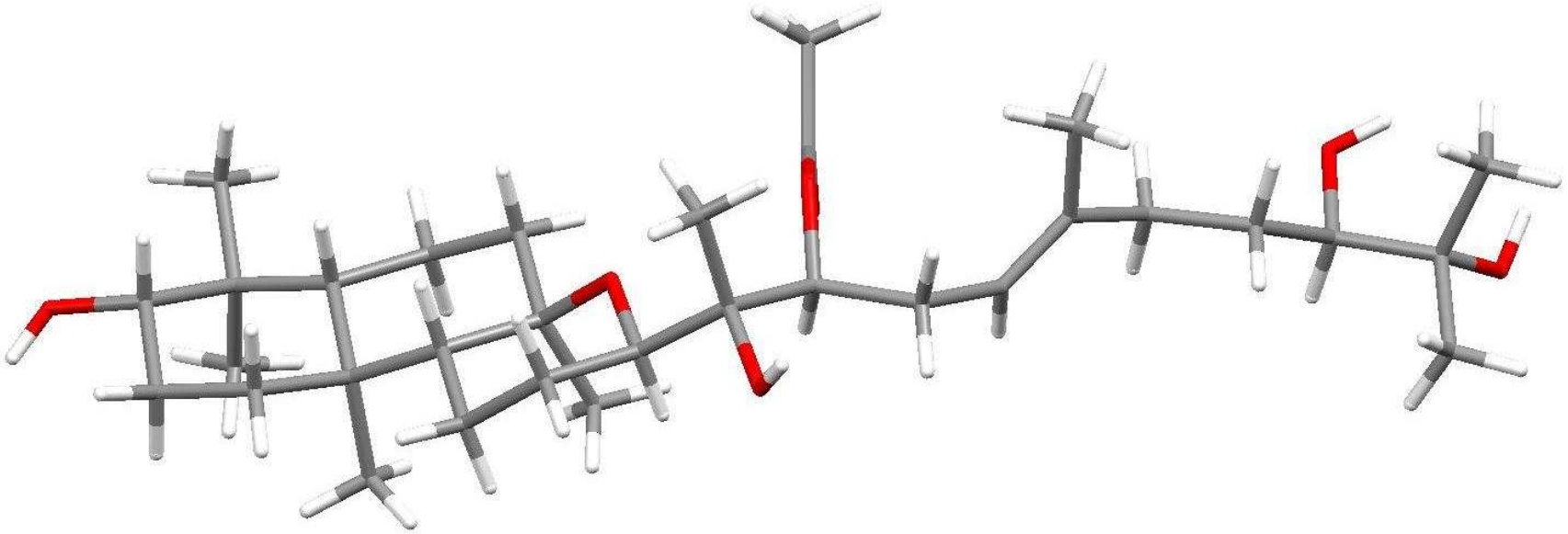

Figure S13. X Ray Structure of Phyteumoside B (**2**) (Capped Stick Drawing).

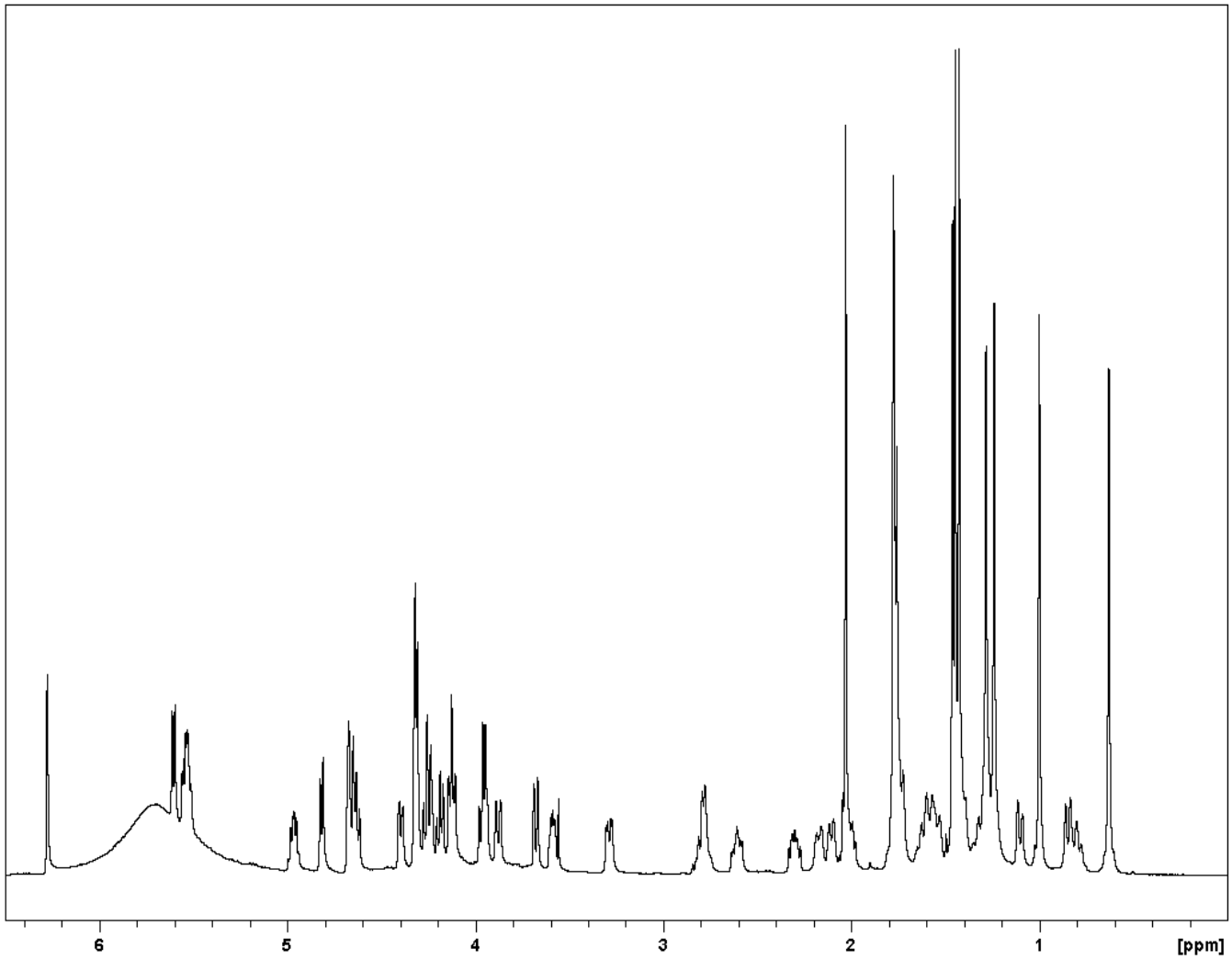

Figure S14. ^{1}H NMR Spectrum of Phyteumoside B (**2**).

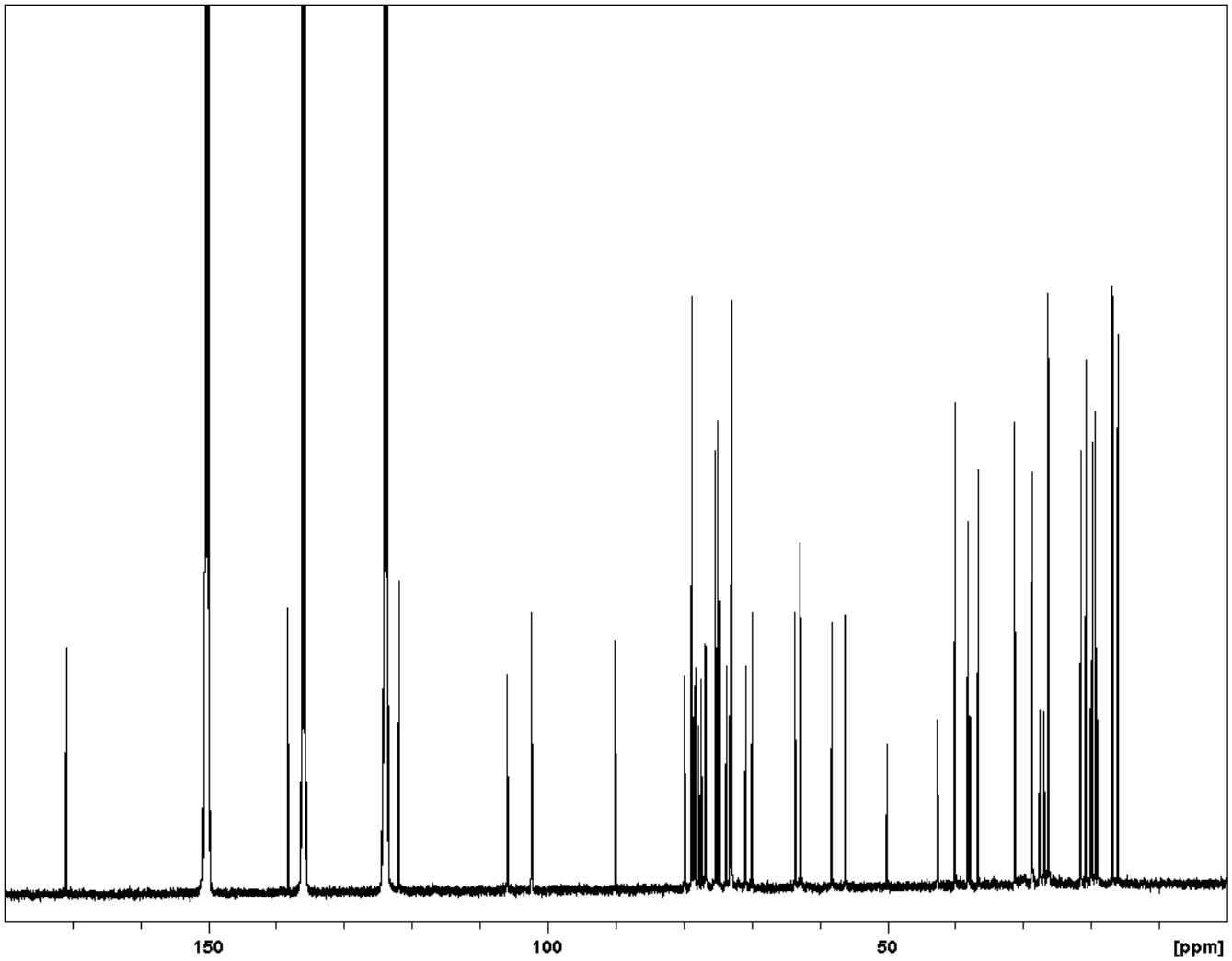

Figure S15. ^{13}C NMR Spectrum of Phyteumoside B (2).

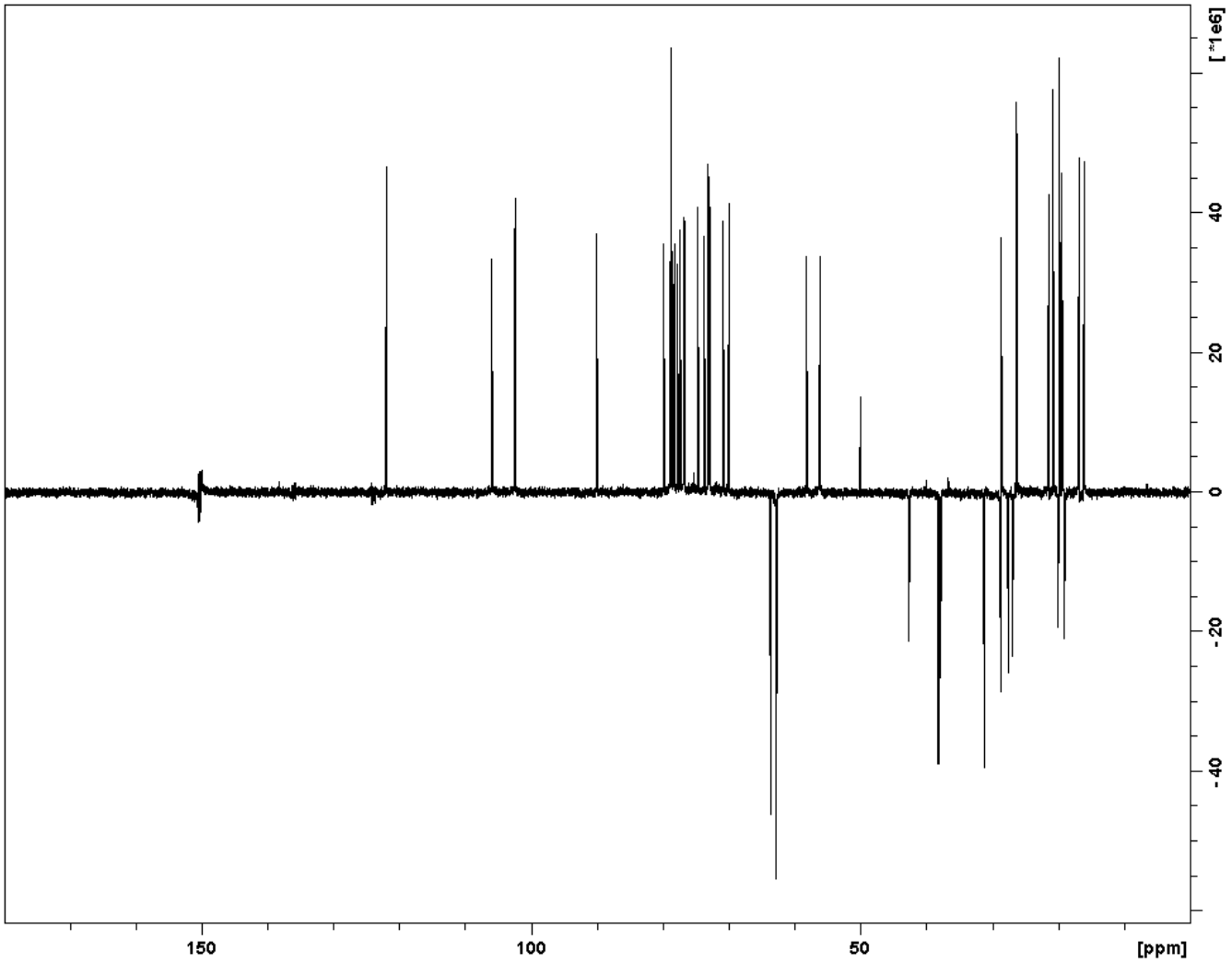

Figure S16. DEPT Spectrum of Phyteumoside B (2).

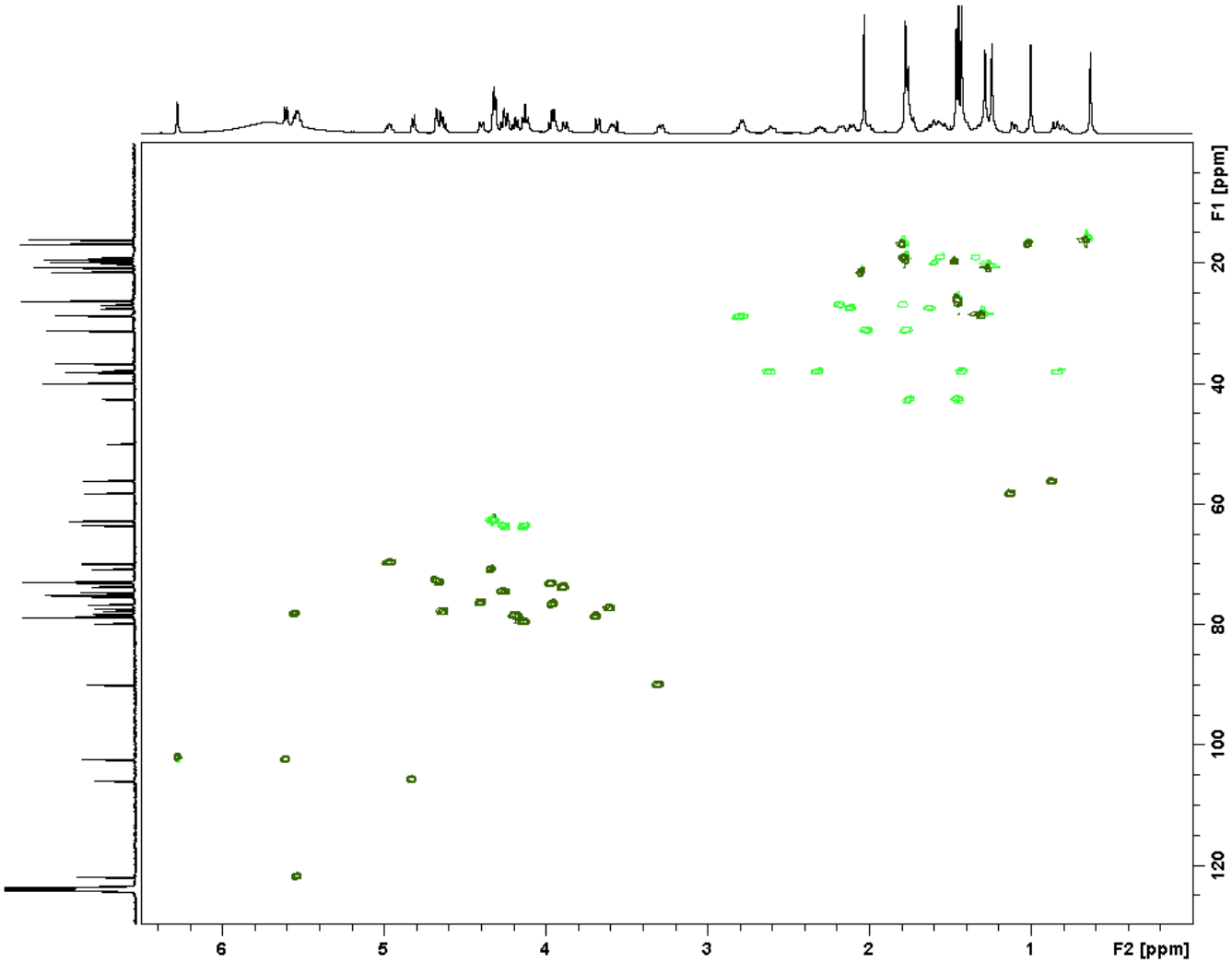

Figure 17. HSQC Spectrum of Phyteumoside B (**2**).

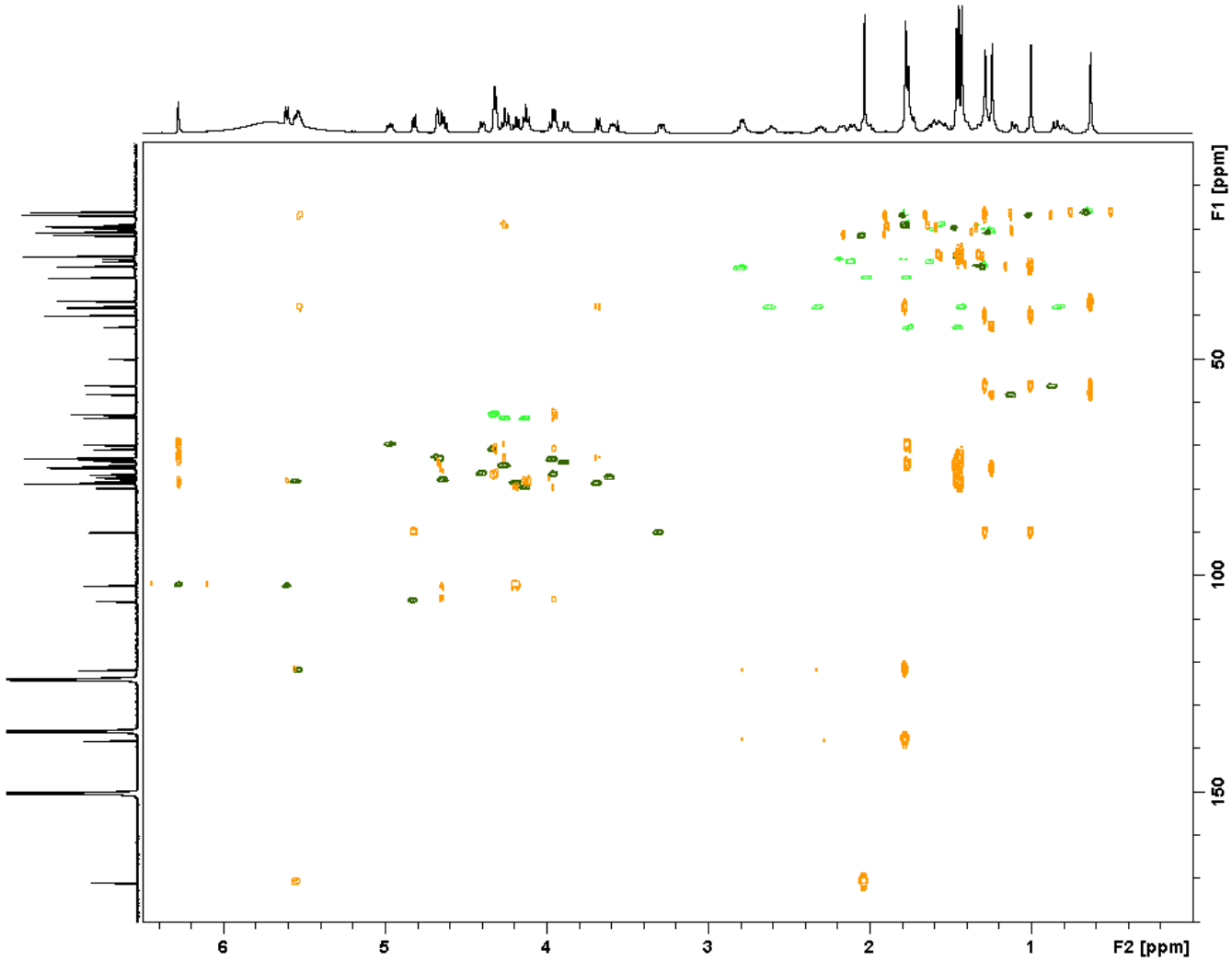

Figure 18. HMBC Spectrum of Phyteumoside B (**2**).

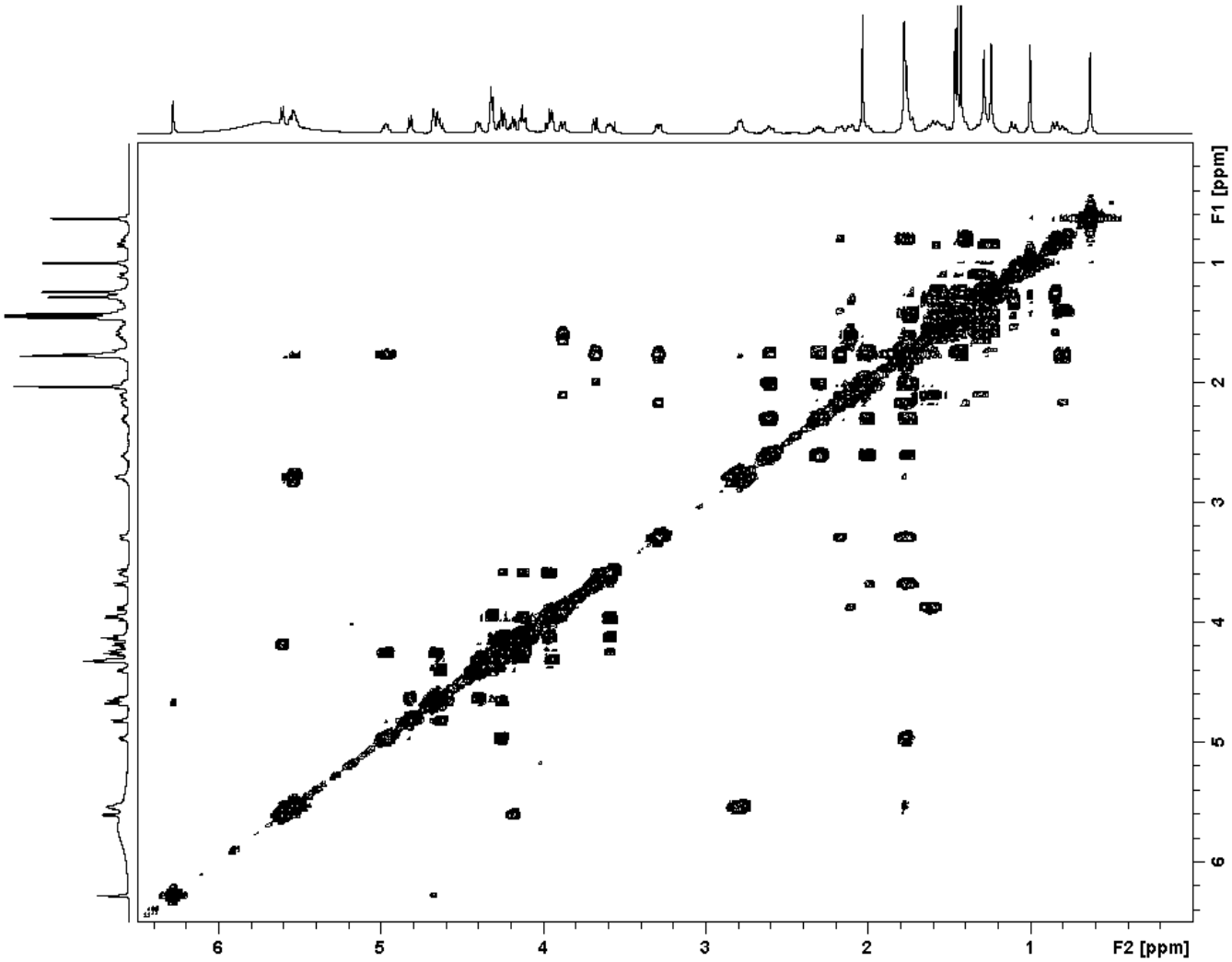

Figure 19. COSY Spectrum of Phyteumoside B (**2**).

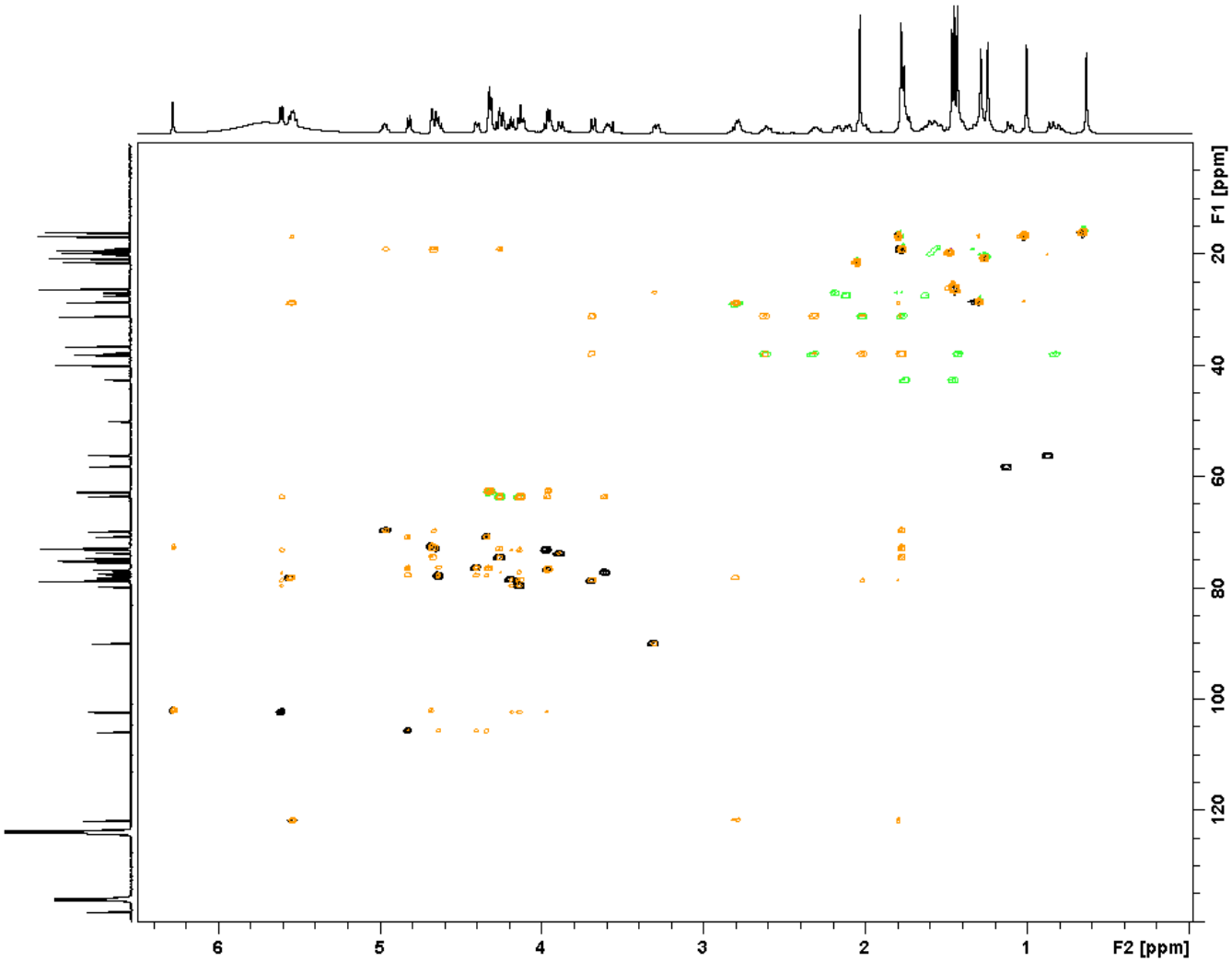

Figure 20. TOCSY Spectrum of Phyteumoside B (**2**).

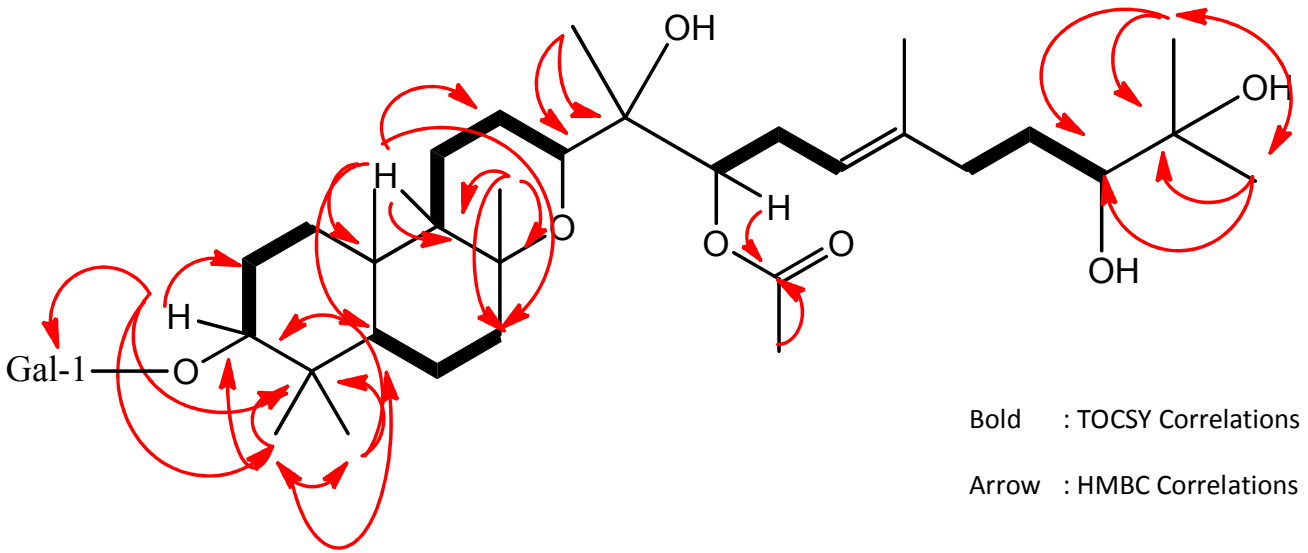

Figure 21. TOCSY and HMBC correlations of Phyteumoside B (**2**).

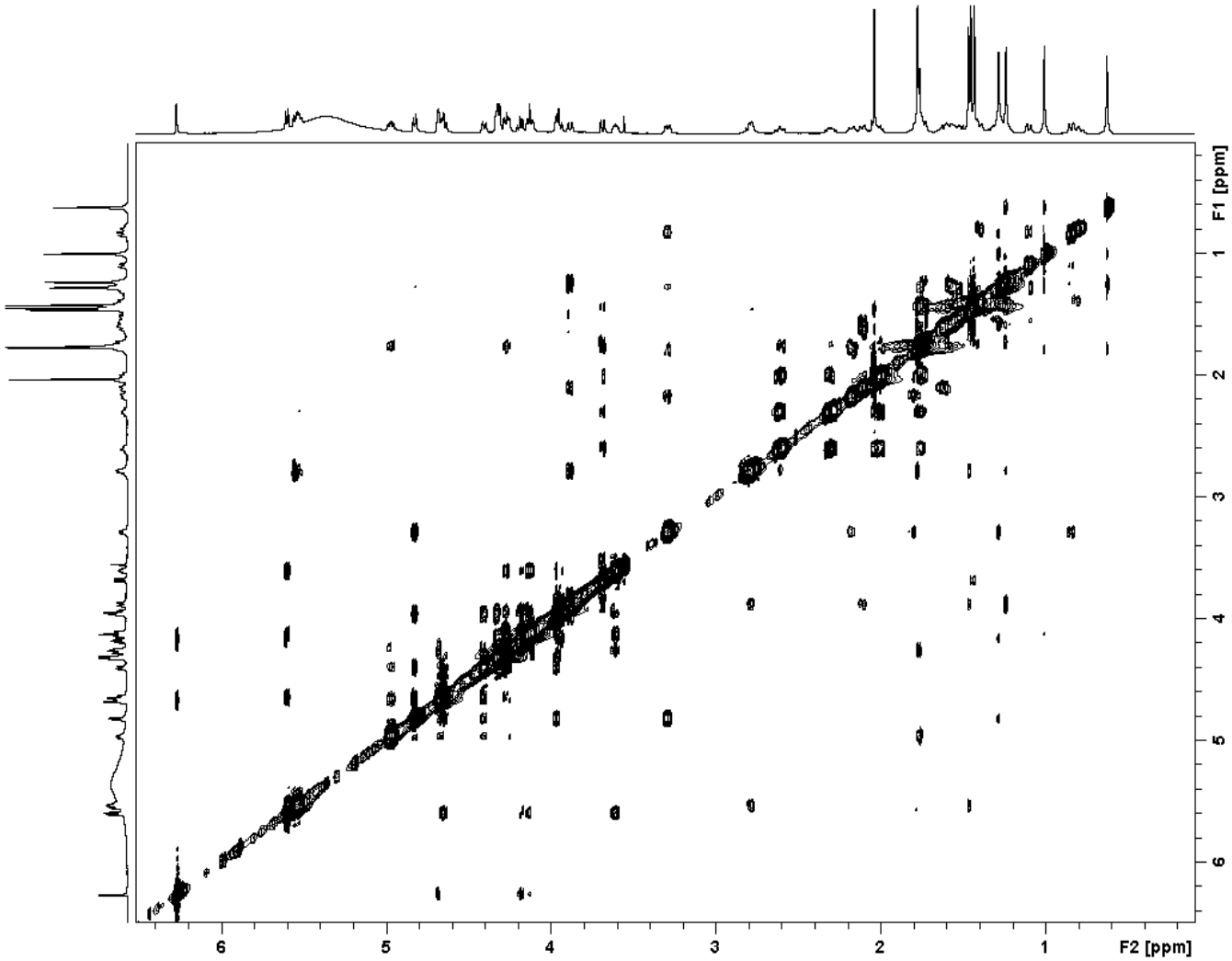

Figure 22. ROESY Spectrum of Phyteumoside B (**2**).

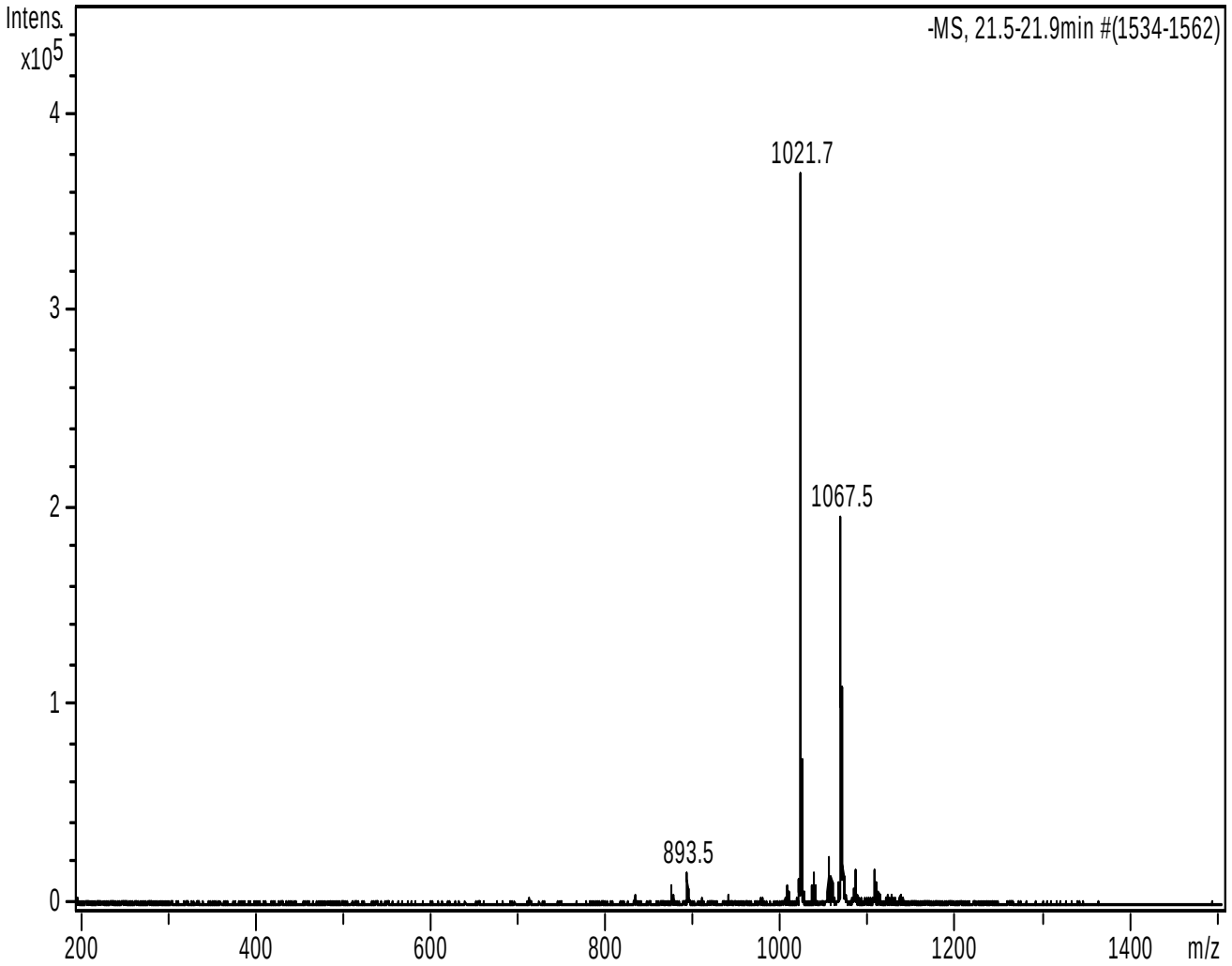

Figure 23. ESI-MS Spectrum of Phyteumoside B (2).

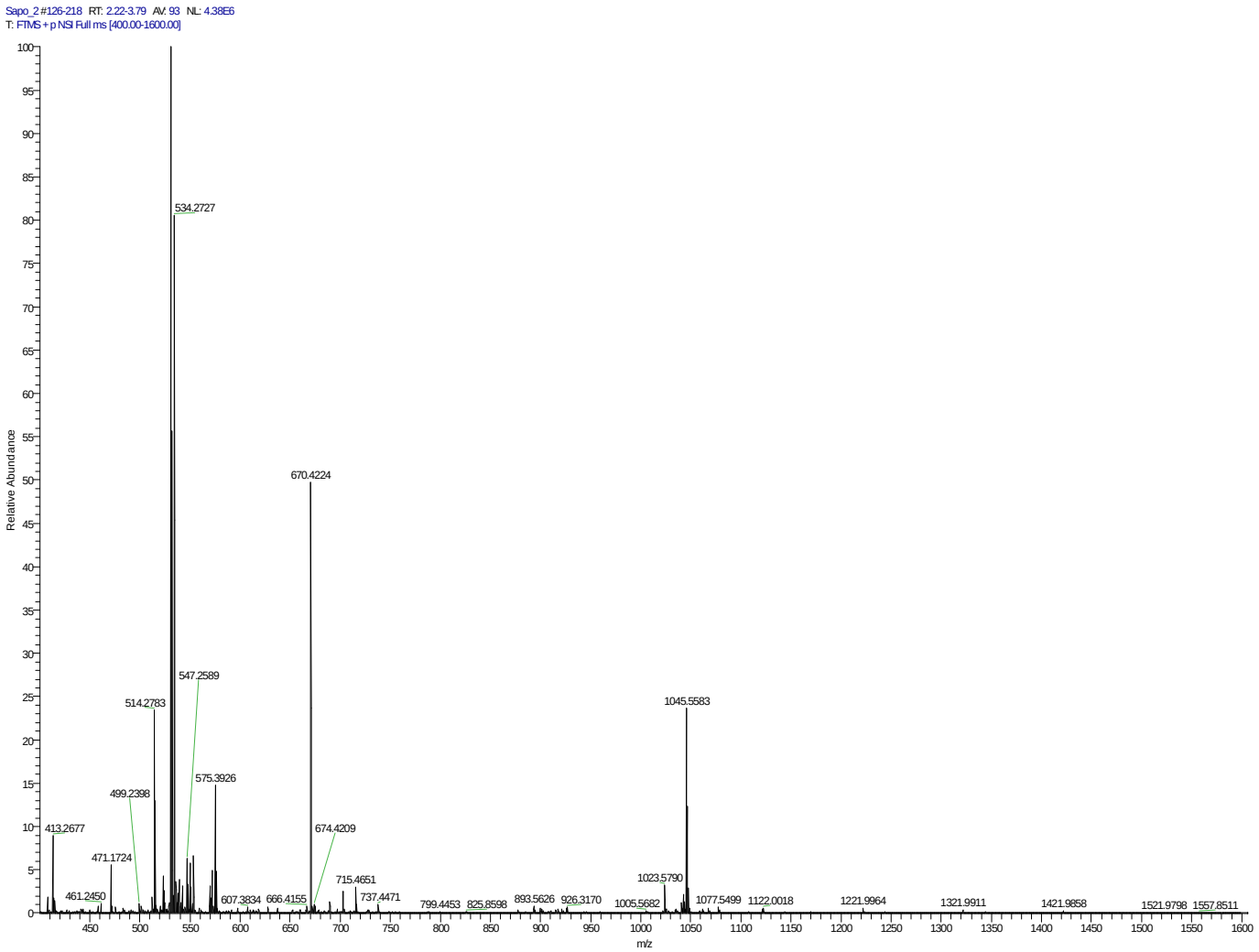

Figure 24. HR-ESI-MS Spectrum of Phyteumoside B (**2**).

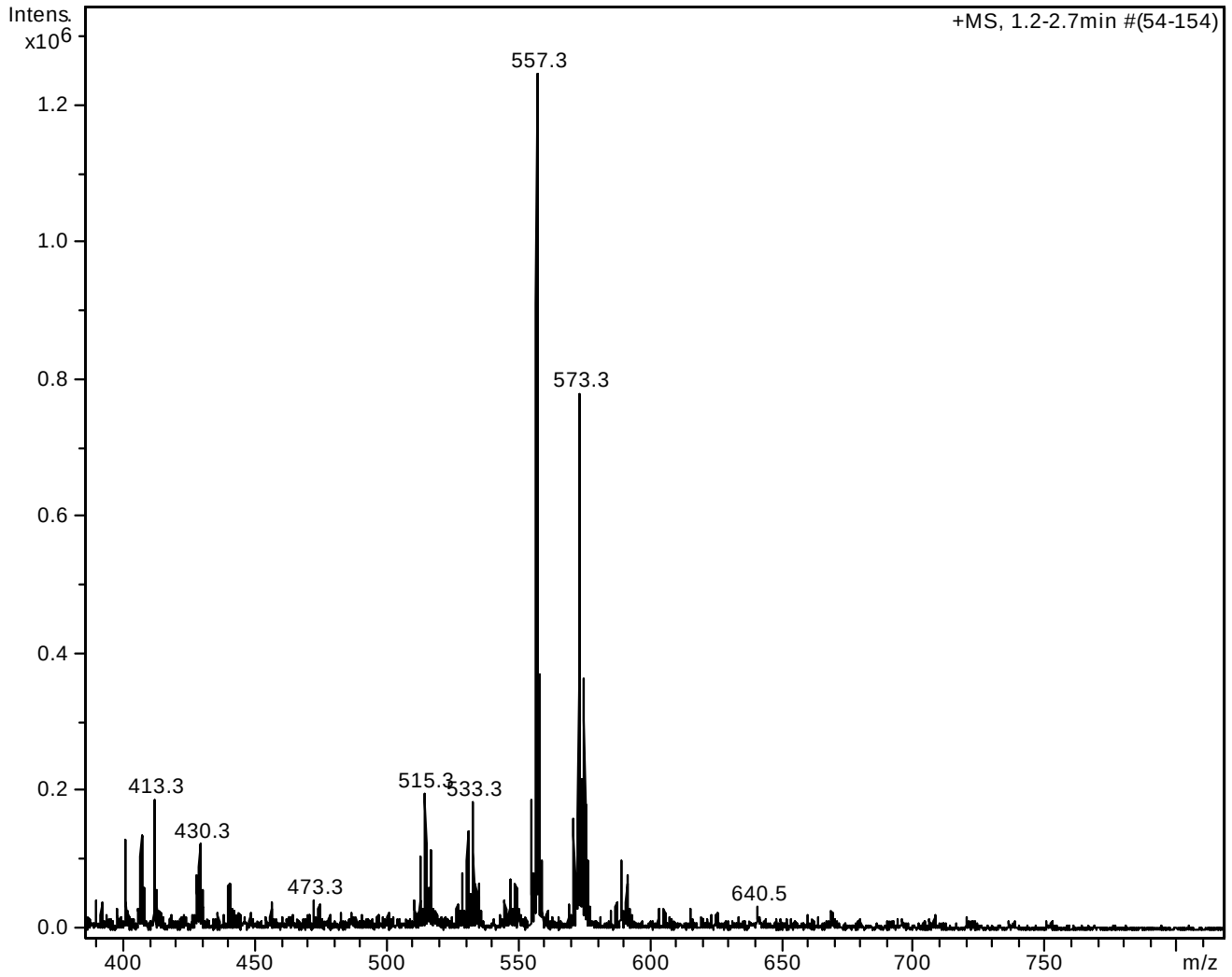

Figure S25. ESI-MS Spectrum of the Aglycon of Phyteumoside A (**1a**).

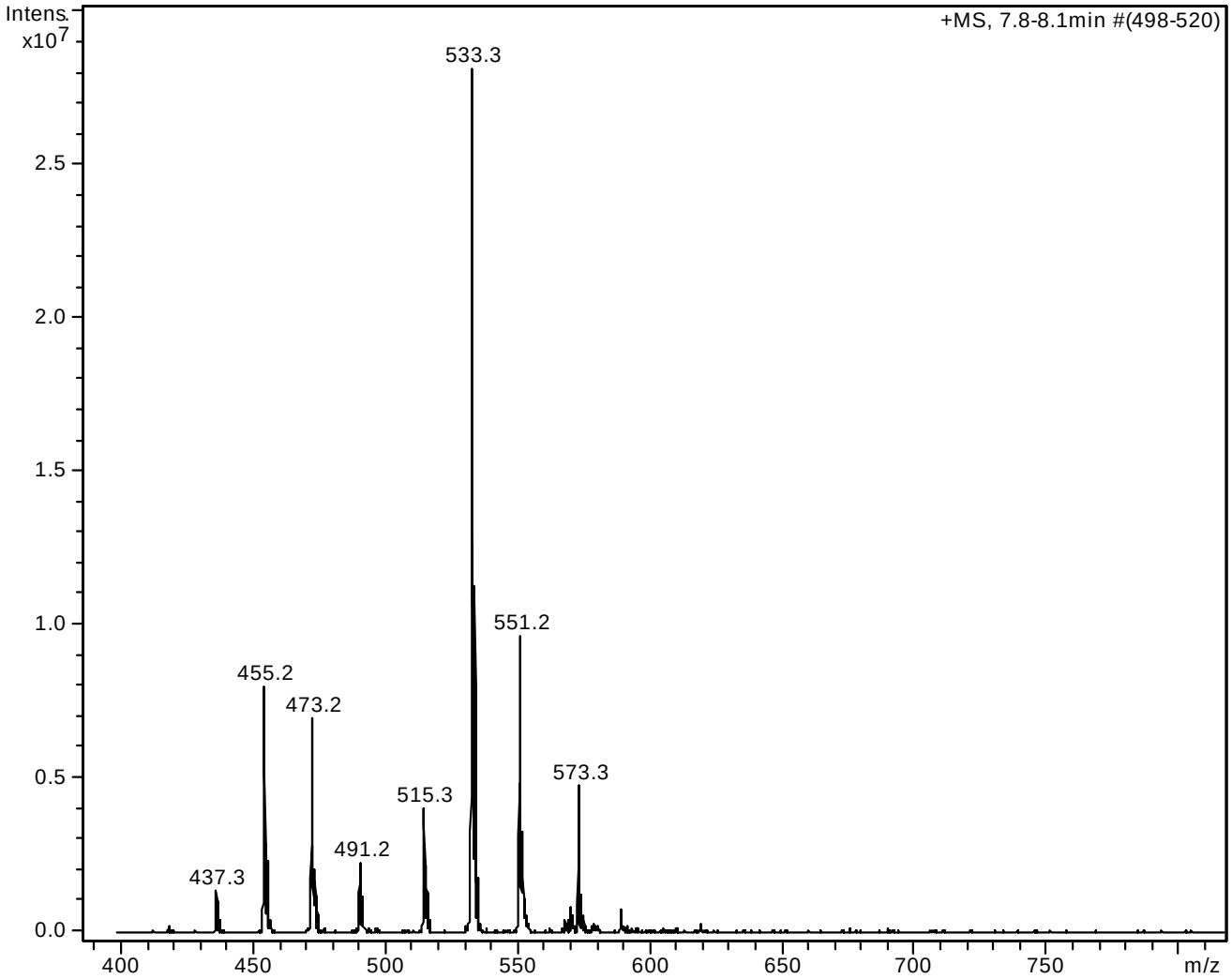

Figure 26. ESI-MS Spectrum of the Aglycon of Phyteumoside B (**2a**).

3.2.2. Comprehensive analysis of *Phyteuma orbiculare* L., a wild alpine food plant

Christian Abbet, Ivan Slacanin, Matthias Hamburger, Olivier Potterat.

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Comprehensive analysis of *Phyteuma orbiculare* L., a wild Alpine food plant

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Dimeric phenylpropanoid glucoside

ABSTRACT

Plants which have been traditionally eaten by the alpine population may provide new opportunities for agricultural development in mountain regions. In this context we have investigated the chemical composition of *Phyteuma orbiculare* (Campanulaceae), a perennial herb whose leaves have been eaten as salad by rural populations in Valais (Switzerland). Extracts of different polarities were subjected to comprehensive metabolite profiling using a dereplication platform combining HPLC–PDA-MS, and offline NMR analysis. Twenty-three compounds, including various phenolic glycosides, a new dimeric phenylpropanoid glucoside, saponins, and fatty acids were identified online, or after targeted isolation. Selected phenolic constituents were quantitatively assessed by HPLC–PDA analysis. In addition, substances relevant for nutrition, such as β-carotene, fatty acids, ascorbic acid and minerals were quantified in leaves and flowers. The antioxidant capacity was determined with an ORAC assay, and total phenolic compounds were quantified. Finally, the phytochemical profile was compared to that of the related species *P. spicatum*, *P. hemisphaericum* and *P. ovatum*.

1. Introduction

The alpine regions of Europe have a rich ancestral tradition with regard to the consumption of wild plants as medicines or food (Pieroni & Giusti, 2009). However, knowledge about these plants and their uses tends to become lost as much of it has been transmitted through oral traditions. Ancient food plants with interesting gustatory properties may represent interesting opportunities for development of mountain agriculture if they can be suitably taken into cultivation. Rocket salad (*Eruca sativa*) is an example of the successful development from a locally used food plant into a mainstream salad (Sastry, 2003). However, prior to any considerations regarding possible cultivation and development, a comprehensive chemical analysis of such plants is required as the basis for an assessment of their nutritional value and safety.

We recently carried out an ethnobotanical survey in Lower and Central Valais (Switzerland) of traditionally used fruits, vegetables and spices. During semi-structured interviews with informants, we established that various species from the genus *Phyteuma* (Campanulaceae) have been traditionally used as edible plants. The genus *Phyteuma,* commonly known as rampion, is native to Europe and Western Asia and contains about 40 perennial herbs (Shulkina, Gaskin, & Eddie, 2003) including the commonly occurring species *P. orbiculare* L. and *P. spicatum* L. The leaf rosettes of both species are characterised by a pleasant nutty taste, and have been used until recently as salad by the rural Alpine population. In addition, the flowers were eaten as sweets by young shepherds. Despite a broad geographic distribution, no information was available on the chemical constituents of the entire genus *Phyteuma* when we began our investigations. In a first contribution, we reported the isolation and the structure elucidation of two triterpene saponins with structurally unique aglycons from the aerial parts of *P. orbiculare* (Abbet et al., 2011). In a continuation of our investigation, we now report a comprehensive phytochemical profiling of the aerial parts of this plant. In addition, we provide data from a quantitative analysis of nutritionally valuable substances, such as ascorbic acid, β-carotene, minerals, and fatty acids. The antioxidant capacity was measured with an ORAC (oxygen radical absorbance capacity) assay and the phenolic compounds were quantified in the leaves and flowers. Finally, the phytochemical profiles of leaves and flowers of *P. orbiculare* were separately investigated with the aid of HPLC–PDA-ESIMS analyses, and compared to those of three further *Phyteuma* species, *P. spicatum* L., *P. ovatum* L., and *P. hemisphaericum* L.

2. Material and methods

2.1. Plant material

The aerial parts of *Phyteuma orbiculare* L. (Voucher nr. 532) used for metabolite profiling and compound isolation were collected by C. Abbet on 24th June 2009 in l'Amônaz, near Orsières, Valais,

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Switzerland. The leaves and flowers of *P. spicatum* L. (Nr. 794), *P. ovatum* L. (Nr. 795), and a second batch of *P. orbiculare* (Nr. 799) with leaves and flowers separately harvested and used for comparative studies were collected on 11th June 2011 in Ferret, near Orsières, Valais, Switzerland. *P.hemisphaericum* L. (Nr. 796) was collected on 11th June 2011 in la Breya, near Orsières. The plants were identified by C. Rey, Senior Scientist at the Agroscope Changins-Wädenswil ACW research station in Conthey, Switzerland. Voucher specimens are kept at the Division of Pharmaceutical Biology, University of Basel, Switzerland.

2.2. Chemicals and reference compounds

Solvents were from Scharlau. Technical grade solvents were used after redistillation for extraction and column chromatography (CC). HPLC grade solvents were employed for HPLC. HPLC grade water was obtained by an EASY-pure II (Barnstead, Dubuque IA, USA) water purification system. Deuterated solvents were purchased from Armar Chemicals (Döttingen, Switzerland). Other chemicals used were analytical grade. Linoleic acid (**20**), α-linolenic acid (**22**), palmitic acid (**23**), adenosine (**1**), chlorogenic acid (**2**) and quercetin-3-*O*-β-glucoside (**9**) were purchased from Sigma–Aldrich (Buchs, Switzerland). Ursolic acid (**21**) was obtained from Extrasynthese (Genay, France).

2.3. General experimental procedures

Sephadex LH-20 was purchased from GE Healthcare. Diaion HP-20 resin was obtained from Sigma–Aldrich. Silica gel (0.063–0.200 mm) was purchased from Merck. Flash chromatography was performed on a Sepacore® chromatography system (Büchi Labortechnik, Flawil, Switzerland) equipped with a pre-packed RP-18 cartridge (40 × 150 mm, 40–63 μm, Büchi Labortechnik). Semi-preparative HPLC was performed on an Agilent 1100 series instrument equipped with a PDA detector. Separations were carried out at 25 °C on a SunFire C_{18} column (5 μm, 150 × 10 mm i.d., Waters, Milford, MA, USA) equipped with a precolumn (10 × 10 mm i.d.). Gradients of acetonitrile and water were used; the flow-rate was 4 mL/min. For the purification of **16–19**, an Esquire 3000 plus mass spectrometer (Bruker Daltonics, Bremen, Germany) combined with a Quick Split flow splitter (Analytical Scientific Instruments, split ratio 200:1) was used. A make-up flow was delivered to the MS-line (0.5 mL/min) by an HPLC pump (Young Lin). Pressurized liquid extraction (PLE) was carried out on an ASE 200 instrument (Dionex, Sunnyvale, CA, USA) in 22 mL steel cartridges; preheat time of 1 min, 100% cell volume flush, 80 s purge with nitrogen, pressure 120 bar.

NMR spectra were recorded on a 500 MHz Avance III™ spectrometer (Bruker BioSpin) equipped with a 1-mm TXI microprobe (^{1}H- and 2D-NMR) or a 5-mm BBO probe (^{13}C-NMR). Chemical shifts are reported as δ in ppm with residual solvent signal as internal reference; *J* in Hz. Standard pulse sequences of the software package Topsin 2.1 were used. Optical rotation was measured on a JASCO P-2000 automatic digital polarimeter. UV spectra were recorded on an Ultrospec 210 pro spectrophotometer (Amersham Biosciences, New Jersey, USA). Electrospray mass spectroscopy (ESIMS) and high resolution electrospray mass spectroscopy (HRESIMS) spectra were recorded on Esquire 3000 plus and MicrOTOF mass spectrometers (Bruker Daltonics), respectively.

2.4. Extraction and isolation

The dried aerial parts were ground using a ZM 1 ultracentrifugal mill (Retsch, Haan, Germany), with 0.75 mm Conidur sieve. Powdered aerial parts (226 g) were extracted at room temperature with dichloromethane (3 × 2 L, each 24 h) followed by methanol (3 × 2 L, each 24 h). The extracts were evaporated to dryness under reduced pressure to obtain 11.9 g of dichloromethane extract, and 42.3 g of methanol extract.

A portion (10.3 g) of the dichloromethane extract was loaded onto a silica gel column (100 × 6 cm i.d.) eluted with a gradient of *n*-hexane–ethyl acetate–methanol to give 16 fractions (Fr. 1–16). After liquid/liquid partition of Fr. 13 (600 mg) between *n*-hexane and methanol, a portion (20 mg) of the *n*-hexane fraction (324 mg) was purified by semi-preparative HPLC on RP-18 with a gradient of 10 to 100% acetonitrile in 30 min, to afford compounds **18** (1 mg) and **19** (1 mg).

A portion (42.3 g) of the methanol extract (43.7 g) was dissolved in 100 mL water and then loaded onto a Diaion HP-20 column (70 × 400 mm i.d.) eluted with water (7 L), followed by methanol (15 L). A portion (5.0 g) of the methanol fraction (6.4 g) was separated on a Sephadex LH-20 column (7 × 100 cm i.d.) eluted with methanol. 15 fractions (Fr. 1–15) were collected. Fr. 3 (263 mg) and 4 (539 mg) were submitted to flash chromatography on RP-18 with methanol–water (20–100%) as eluent to give **14** (40 mg), and **15** (51 mg), respectively. Frs. 11, 12, 14 and 15 were purified by semi-preparative HPLC on RP18 with methanol–water gradients (see Supplementary Information Data D1). 250 mg of Fr. 11 (849 mg) gave compounds **2** (1 mg), **3** (3 mg), and **4** (5 mg). Likewise, compounds **3** (3 mg) and **7** (2 mg) were isolated from 51 mg of Fr. 12 (101 mg). Compounds **6** (5 mg), **12** (2 mg), and **13** (2 mg) were obtained after purification of 40 mg of Fr. 14 (96 mg). Finally, 42 mg of Fr. 15 (63 mg) provided compounds **1** (6 mg), **6** (3 mg), **9** (4 mg), and a mixture of two compounds (14 mg). They were separated by Sephadex LH-20 CC (40 × 1 cm i.d.) with methanol, to obtain **10** (6 mg), and **12** (5 mg). Compound **11** (6 mg) was isolated from 100 mg of Fr. 6 (213 mg), after separation by silica gel CC with a gradient of *n*-hexane–ethyl acetate–methanol. Fraction 10 (418 mg) was separated by silica gel CC eluted with a gradient of ethyl acetate–methanol. Semi-preparative HPLC on RP18 with a gradient of 10 to 50% acetonitrile in 30 min afforded compounds **16** (2 mg), and **17** (4 mg).

Tangshenoside VII (**8**): White amorphous powder. $[\alpha]^{20}_D$ −19.4 (*c* 0.05, MeOH). UV (MeOH) λ_{max} (log ε): 220 (4.03), 268 (3.72). ESIMS: *m/z* 1337 $[M–H]^-$, 1361 $[M + Na]^+$. ESIMS^2 (1337): *m/z* 983 [M-Glc-dimethoxyhydroxybenzylidene]$^-$, 839 [M-ussurienoside]$^-$, 659 [M-ussurienoside-Glc]$^-$. HR-ESIMS: *m/z* 1361.4478 $[M + Na]^+$ (calc. for $C_{58}H_{82}NaO_{35}$ 1361.4534). ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2.

2.5. HPLC–PDA-MS analyses

HPLC–PDA-MS analyses were carried out on an Agilent series 1100 system consisting of a binary pump, a column oven and a photodiode array (PDA) detector (Agilent Technologies, Waldbronn, Germany), connected to a 215 injector (Gilson, Mettmenstetten, Switzerland) and to an Esquire 3000 plus ion trap mass spectrometer equipped with electrospray (ESI) interface (Bruker Daltonics). Separations were performed on a SunFire C_{18} column (3.5 μm, 150 × 3.0 mm i.d., Waters) equipped with a guard column (20.0 × 3.0 mm i.d.). The mobile phase consisted of water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B). Gradients of 5–50% B in 30 min, then to 100% B in 5 min (methanol extract) or 10–100% B in 30 min, then 100% B during 5 min (dichloromethane extract) were used. The flow rate was 0.4 mL/min, and the column temperature was set to 25 °C. 20 μL of a 5 mg/mL (extracts), or a 1 mg/mL (pure compounds) solution were injected. UV spectra were recorded from 200 to 400 nm. ESIMS spectra were recorded in the negative ion mode under ion charge conditions (ICC 20000), at a scan speed of 13000 *m/z*/s, using a gauss filter width of 0.2 *m/z*. Nitrogen was used as drying gas at a flow rate of 10 L/min and as a nebulizing gas at a pressure of 30 psi. The nebulizer temperature was set at 300 °C. Spectra were recorded in the

range of *m/z* 100 to 1500. Capillary voltage set at −4500 V, endplate offset at 500 V, capillary exit at −128.5 V, skimmer voltage at −40 V, and trap drive at 61.4 V. Data acquisition and processing were performed using Hystar 3.0 software (Bruker Daltonics).

2.6. Comparative analyses of different Phyteuma species

Separately harvested leaves and flowers of *P. orbiculare*, *P spicatum*, *P. hemisphaericum* and *P. ovatum* were immediately shock frozen in dry ice. The plant material was freeze-dried and milled. A 2.1 g portion of each sample was extracted by pressurized liquid extraction (PLE) successively with dichloromethane and methanol (70 °C, 3 extraction cycles of 5 min). HPLC analysis was performed as described in 2.5.

2.7. Fatty acid composition

Freeze dried leaves or flowers (2.0 g) of *P. orbiculare* were extracted separately by PLE with *n*-hexane–isopropanol (9:1) (70 °C, 3 extraction cycles of 3 min each). The extract was adjusted to a final volume of 50.0 mL with *n*-hexane–isopropanol (9:1). The method of the European Pharmacopeia V was used for analysis (European Pharmacopeia V., 2005). An aliquot of extract (15.0 mL) was mixed with 2.0 mL of internal standard solution (5.0 mg/mL of methyl tricosanoate (NU-Chek Prep, Elysian, MN) in isooctane) in a quartz tube and the solvent was evaporated under nitrogen. 1.5 mL of a 20 g/L solution of NaOH in methanol was added. The tube was filled with nitrogen and sealed with a polytetrafluoroethylene-lined cap. The reaction mixture was heated at 90 °C for 7 min. After cooling, 2 mL of BF_3-methanol (12:88) were added, the mixture heated again at 90 °C under nitrogen for 20 min, then cooled down to room temperature. 1.5 mL of isooctane was added and the mixture shaken vigorously. Following the addition of 5 mL of a saturated NaCl solution, the mixture was shaken again and centrifuged for 5 min at 5000 rpm. The upper layer was transferred into a new tube, and the lower layer was extracted under shaking with 1.5 mL of isooctane. The combined isooctane extracts were washed with 1 mL of water. 1 μL was analysed by GC-FID on a polyethylene glycol INNOVAX (30 × 0.32 mm, 0.25 μm film thickness) column with a temperature gradient of 3 °C/min from 170 to 240 °C (helium as carrier gas at a flow rate of 1.9 mL/min, split ratio (1:50)). The temperatures of injector and detector were 250 and 280 °C, respectively. Analyses were performed in triplicate. The concentrations of fatty acids are expressed as percent fresh weight by taking into account the moisture determined for leaves (40.9%) and flowers (70.7%).

2.8. Determination of β-carotene

Freeze-dried leaves or flowers (each 1.0 g) were separately extracted by PLE with *n*-hexane–isopropanol (9:1) (70 °C, 3 extraction cycles of 3 min each). The extracts were analysed by HPLC on an Agilent series 1200 system consisting of a binary pump, a column oven and a photodiode array (PDA) detector. Separations were performed on a Nucleosil 120–5 C_{18} (5 μm, 250 × 3 mm i.d.) (Macherey Nagel) column with methanol–acetonitrile-tetrahydrofuran (91:5:4) at a flow rate of 1.0 mL/min. Detection was at 452 nm. Analyses were performed in triplicate. The concentration of β-carotene (including isomers) is expressed as percent fresh weight by taking into account the moisture determined for leaves (40.9%) and flowers (70.7%).

2.9. Determination of ascorbic acid

The method described in the *Manuel Suisse des Denrées Alimentaires* (MSDA) was used with slight modification (MSDA. Manuel Suisse des Denrées Alientaires, 1992). Fresh leaves or flowers (about 5.0 g exactly weighed) were mixed with quartz sand and ground in a mortar with 40.0 mL 5% metaphosphoric acid for 5 min. The mixture was filtered through a 0.25 μm PTFE filter and the filtrate used for quantitative determination. Analyses were carried out on an Agilent series 1200 system consisting of a binary pump, a column oven and a photodiode array (PDA) detector. Separations were performed on an EC Nucleosil C18 column (100–5 μm, 250 × 4.0 mm i.d., Macherey–Nagel) equipped with a guard column (8.0 × 4.0 mm i.d.). The mobile phase consisted of water containing 1.03 g/L of *n*-hexane sodium sulfonic acid (pH adjusted to 2.6 with 40% H_3PO_4) (solvent A) and acetonitrile–water (8:2) (solvent B). A gradient elution was used with 0, 10, 20, 22, 28 and 40% B at 0, 5, 12, 15, 20 and 23 min, respectively. The flow rate was 1 mL/min, and the column temperature was set to 25 °C. 10 μL were injected. Detection was at 241 nm. The limits of detection (LOD) and quantification (LOQ) were 0.5 mg and 2.0 mg/100 g FW, respectively. Analyses were performed in triplicate.

2.10. Determination of minerals

A standard operation procedure of the University of Wisconsin, Madison, was used with minor modifications (University of Wisconsin-Madison., 2005). Fresh leaves or flowers (1.0 g) were mixed with 10 mL ultrapure concentrated HNO_3 and 1 mL of 30% H_2O_2. The mixture was shaken in a PTFE tube for 6 h at 60 °C. After centrifugation at 3000 rpm for 5 min., the supernatant was adjusted to 20.0 mL with HNO_3 30% and finally diluted 20 times with 30% HNO_3. Minerals were quantified with an inductively coupled plasma emission spectrometer (ICPE 9000, Shimadzu, Kyoto, Japan). The following parameters were used: power 1200 W; plasma view: axial; nebulization gas: argon, 0.7 L/min; shear gas: argon, 15 L/min; auxiliary gas: argon, 0.3 L/min. The detection limit (LOD) of each element was 0.3 mg/100 g FW. Analyses were performed in triplicate.

2.11. Total phenolic content

Frozen flowers and leaves (each 200 mg) were separately crushed with 2 mL 50% aqueous methanol in a nitrogen-cooled mortar. The homogenate was centrifuged (3000*g* for 15 min at 4 °C). The determination of total phenolics was done following the standard Folin–Ciocalteau (FC) method described in the Current Protocols in Food Analytical Chemistry (Waterhouse, 2002). Briefly, 20 μL of each sample, gallic acid standards (250–1500 mg/L) or blank (water) were mixed with 1580 μL of water and 100 μL FC solution (Sigma). The mixture was incubated for 4 min at room temperature. Then, 300 μL of a saturated sodium carbonate solution were added and the solution was incubated for two hours in the dark at room temperature. 200 μL of each reaction mixture were transferred into a 96 well microplate, and the absorbance was measured at 750 nm on a Chameleon multilabel detection platform (Hidex, Turku, Finland). The absorbance recorded for the gallic acid standards ($n = 3$) was plotted as a function of concentration. The calibration curve ($y = 0.0006$ x + 0.0168 ($r^2 = 0.9931$)) was then used to determine the number of gallic acid equivalents (GAE) in the samples ($n = 4$).

2.12. Quantitative determination of selected phenolic constituents

Leaves and flowers of *P. orbiculare* were freeze-dried and milled. 2.1 g of each were separately extracted by pressurized liquid extraction (PLE) with dichloromethane and methanol, successively (70 °C, 3 extraction cycles of 5 min). HPLC analyses were performed in triplicate on an Alliance 2695 instrument (Waters) equipped with a 996 PDA detector. Separations were performed

on a SunFire C_{18} column (3.5 μm, 150 × 3.0 mm i.d., Waters) equipped with a guard column (20.0 × 3.0 mm i.d.). The flow rate was 0.4 mL/min. The mobile phase consisted of water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B). A gradient of 5–50% B in 40 min was applied and the column was allowed to equilibrate for 10 min between injections. Injection volume was 10 μL. Samples were dissolved in DMSO at a concentration of 10 mg/mL for extracts and 0.02 to 1 mg/mL for references. Chlorogenic acid and quercetin 3-*O*-β-glucoside were of commercial origin, while luteolin 7-*O*-rutinoside, luteolin 7-*O*-rutinoside- 4′-*O*-β-glucoside and tangshenoside VII were isolated from *P. orbiculare* during this study. Detection was at 267 nm for tangshenoside VII and 350 nm for the other compounds. Calibration curves were used to determine the concentration of the respective compounds in the extracts. Chlorogenic acid: $3.24 \times 10^7\ x - 3.13 \times 10^5$ ($r^2 = 0.9999$); luteolin 7-*O*-rutinoside: $y = 1.41 \times 10^7\ x + 1.39 \times 10^5$ ($r^2 = 0.9991$); luteolin 7-*O*-rutinoside, 4'*O*-β-glucoside: $y = 4.34 \cdot 10^7\ x + 1.70 \times 10^5$ ($r^2 = 0.9979$); quercetin 3-*O*-β-glucoside: $y = 4.11 \times 10^7\ x - 7.81 \times 10^4$ ($r^2 = 0.9979$); tangshenoside VII: $y = 1.87 \times 10^7\ x + 6.74 \times 10^3$ ($r^2 = 0.9996$).

2.13. Oxygen radical absorbance capacity (ORAC)

Frozen flowers and leaves (each 100 mg) were separately crushed with 10 mL 50% aqueous methanol in a nitrogen-cooled mortar. The homogenate was centrifuged (3000*g* for 15 min at 4 °C). The ORAC assay was performed in a 96 well microplate according to the method described in literature (Gillespie, Chae, & Ainsworth, 2007), with some minor modifications. All solutions were prepared in phosphate buffer (75 mM, pH 7.2). 100 μL of a 0.025 mM fluorescein solution and 50 μL of blank, Trolox® standards (60, 30, 24, 15, 7.5 μM), gallic acid solutions (50, 25, 12.5, 6.25 μM), or 1000-fold diluted extracts were added to each well. The microplate was preincubated for 30 min at 37 °C. The reaction was initiated by the addition of 50 μL of a freshly prepared solution of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) (320 mg in 4.5 mL), and fluorescence was monitored every four minutes using a Chameleon Multilabel Detection Platform (λ_{ex} = 485, λ_{em} = 535 nm). The decrease in fluorescence recorded with Trolox® antioxidant standards (n = 3), gallic acid (n = 3), blanks (n = 3), and samples (n = 4) was plotted using OriginLab software (see Supplementary Information Fig. S8). The area under the curve (AUC) was calculated from the formula (Ou, Hampsch-Woodill, & Prior, 2001):

$AUC = \sum_{i=0}^{n} \frac{f_i}{f_0}$ where f_0 is the fluorescence at $t = 0$, and f_i is the fluorescence at $t = i$.

The net AUC was obtained by subtracting the AUC of the blank from that of the standards or samples. The net AUC of the different Trolox® solutions was plotted as a function of concentration and the curve ($y = 0.1068 \times -0.3062$ ($r^2 = 0.9999$)) used to calculate the antioxidant capacity of the samples to be tested.

3. Results and discussion

3.1. Profiling of the secondary metabolites of P. orbiculare

To obtain a comprehensive phytochemical profile of *P. orbiculare*, the dried aerial parts were extracted successively with dichloromethane and methanol, and both extracts submitted to HPLC–PDA-MS analysis.

HPLC–PDA-MS data of the methanolic extract (Fig. 1) revealed a complex pattern characterised by the presence of major peaks originating from UV-absorbing polyphenolic compounds (Fig. 1A) and compounds without chromophores which were only detected in the MS-trace (Fig. 1B). Two major peaks were assigned to the triterpene saponins phyteumosides A (**15**) and B (**14**) which we recently reported from this species (Abbet et al., 2011). In addition, several compounds, including flavonoid glycosides, various phenolics, a sterol glucoside and fatty acids were identified after targeted purification by a combination of Sephadex LH-20, silicagel CC, and semi-preparative HPLC (Fig. 2). Campesterol-3-*O*-β-glucoside (**11**), adenosine (**1**) (Ciuffreda, Casatii, & Manzocchi, 2007), *p*-hydroxybenzoic acid (**2**), chlorogenic acid (**3**) (De Almeida et al., 1998), luteolin 7-*O*-β-rutinoside-4′-*O*-β-glucoside (**4**) (Osterdahl, 1979), tangshenoside I (**5**) (Song, Chou, Zhong, & Wang, 2008), luteolin 6-*C*-glucoside (**6**) (Leong et al., 2010), luteolin 7-*O*-β-rutinoside (**7**) (Hoffmann & Lunder, 1984), quercetin-3-*O*-β-glucoside (**9**) (Wang, Guo, Wang, Duan, & Du, 2010), quercetin 3-*O*-(6''-*O*-malonyl)-β -glucoside (**10**) (Wald, Wray, Galensa, & Herrmann, 1989), isorhamnetin 3-*O*-β-glucoside (**12**) (Wang et al., 2010), isorhamnetin 3-*O*-(6″-*O*-malonyl)-β-glucoside (**13**) (Wald et al., 1989), 9,12,13-trihydroxy-10,15-octadecaenoic acid (**16**) (Oueslati, Ben Jannet, Mighri, Chriaa, & Abreu, 2006) and 9,12,13-trihydroxy-10,15-octadecadienoic acid (**17**) (Kurashina, Miura, Enomoto, & Kuwahara, 2011) were identified on the basis of UV, ESIMS, ^{1}H- and ^{13}C-NMR data, and by comparison with literature values or reference compounds.

Compound **8** ($[\alpha]^{20}_D$ −19.4 (*c* 0.05, MeOH) proved to be a new secondary metabolite. Its molecular formula was established as $C_{58}H_{82}O_{35}$ from the pseudo-molecular $[M + Na]^+$ ion at *m/z* 1361.4478 (*calc.* 1361.4534) in the HR-ESIMS spectrum. ESIMS^2 experiments on the $[M–H]^-$ pseudomolecular ion at *m/z* 1337 gave fragment ions at *m/z* 983 [M-Glc-dimethoxyhydroxybenzylidene]$^-$, 839 [M-ussurienoside]$^-$, and 659 [M-ussurienoside-Glc]$^-$. The UV spectrum showed absorption maxima at λ_{max} (MeOH) 220 and 268 nm and was almost identical to that of **5**. Comparison of the ^{1}H- and ^{13}C-NMR data of **8** (Table 1) with those of **5** (see Supplementary Information Table S1) indicated that **8** possessed a highly symmetric structure comprising two units of tangshenoside I (3′*O*-β-D-glucopyranosyl-ussurienoside, **5**). HMBC correlation (^3J) observed between H-6″ of glucose (δ_H 4.43 ppm) of one unit and the carboxyl group C-1′ of the hydroxymethylglutaryl (HMG) residue (δ_C 172.6 ppm) of the other unit revealed the linkage between

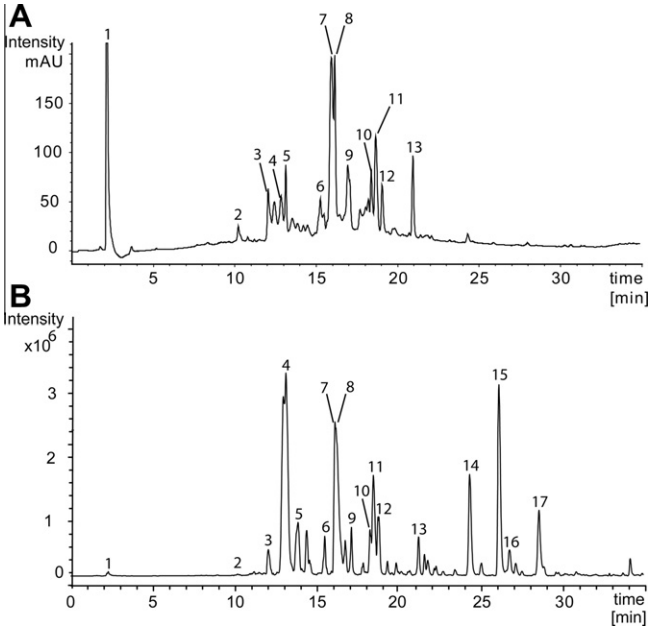

Fig. 1. HPLC–PDA-ESIMS analysis of the methanol extract of dried aerial parts of *P. orbiculare*. (A) UV 254 nm; (B) ESIMS, base peak chromatogram, negative ion mode, *m/z* 150–1500. No additional peaks were detected when the UV trace was recorded at wavelengths down to 220 nm.

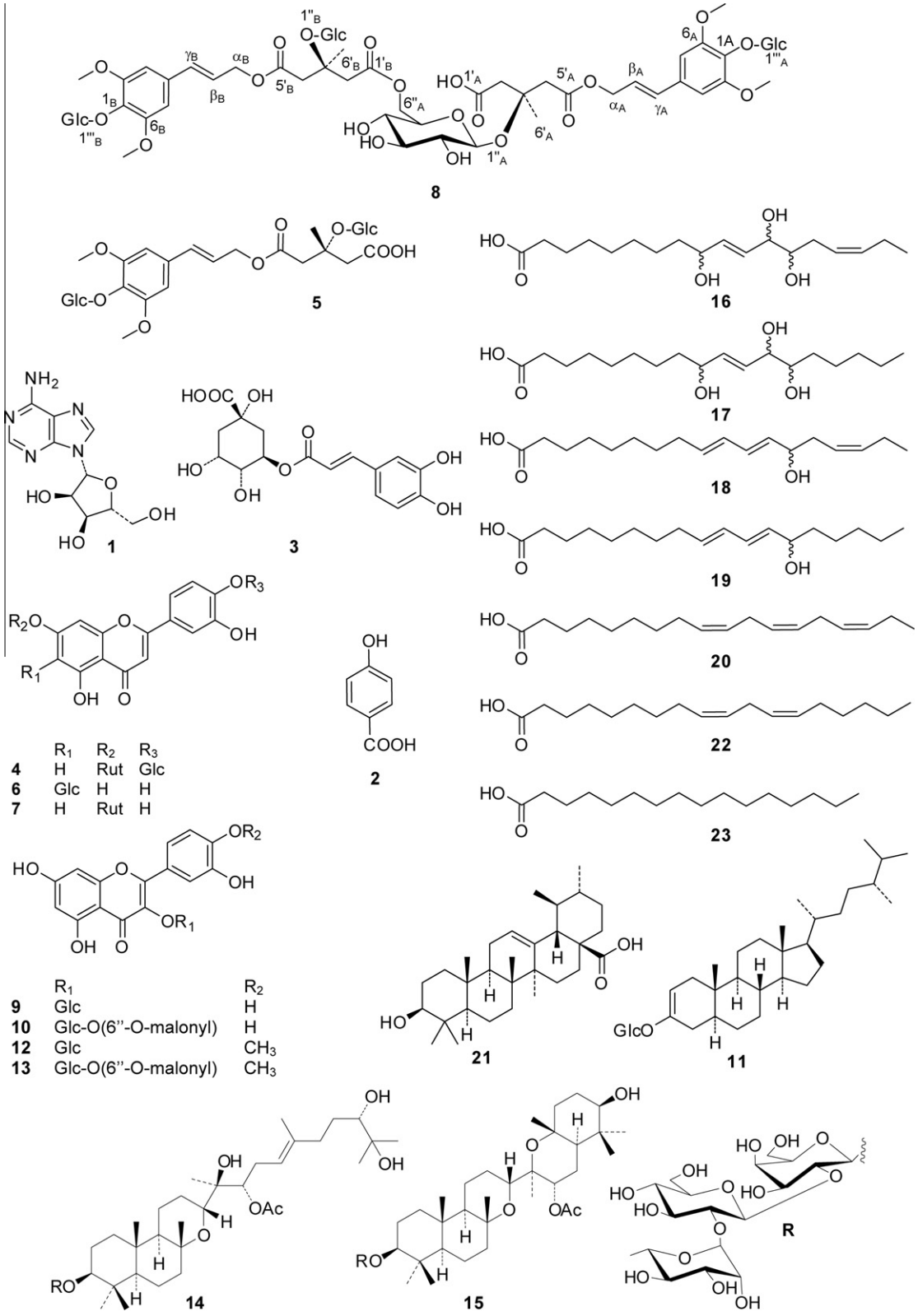

Fig. 2. Structures of the compounds identified in *P. orbiculare*. Glc: glucose, Rut: rutinose.

the two monomeric moieties. Thus, the structure of **8,** for which we suggest the name tangshenoside VII, was assigned as shown in Fig. 2. The configuration of the HMG residues is tentatively proposed as being the same as that reported for the monomer tangshenoside I (**5**) which co-occurs in the plant (Song et al., 2008). Phenylpropanoid derivatives containing a HMG moiety were previously reported from the genera *Campanula* (Cuendet, Potterat, & Hostettmann, 2001), and *Codonopsis* (Song et al., 2008), and thus appear to be quite characteristic for plants of the family Campanulaceae.

Table 1
^{1}H- and ^{13}C- NMR Data[a] of tangshenoside VII (**8**).

Position	δ_H (mult.)[b]	δ_C (mult.)		HMBC[c]	Position	δ_H (mult.)[b]	δ_C (mult.)		HMBC[c]
1_A		134.6	(s)		1_B		134.6	(s)	
2_A–6_A	6.75, s	105.4	(d)	1_A, 3_A, 4_A, 5_A	2_B–6_B	6.71	105.4	(d)	1_B, 3_B, 4_B, 5_B
3_A–5_A		154.4	(d)		3_B–5_B		154.4	(d)	
4_A		136.3	(s)		4_B		136.2	(s)	
α_A	4.73, d (6.1)	66.3	(t)	β_A, γ_A, $5'_A$	α_B	4.73	66.3	(t)	β_B, γ_B, 5_B'
β_A	6.26, ddd (1.0, 6.1, 7.4)	124.5	(d)		β_B	6.29, ddd (1.0, 6.1, 7.4)	124.4	(d)	
γ_A	6.60	135.2	(d)	2_A, α_A, β_A	γ_B	6.63	135.2	(d)	2_B, α_B, β_B
$1'_A$		172.6	(s)		$1'_B$		172.6	(s)	
2_A'	2.76–2.84	44.8	(t)	$1'_A$, $3'_A$	$2'_B$	2.76–2.84	44.5	(t)	$1'_B$, $3'_B$
$3'_A$		76.1	(s)		$3'_B$		76.1	(s)	
$4'_A$	2.87–2.95	44.9	(t)	$3'_A$, $5'_A$, 6_A'	$4'_B$	2.87–2.95	44.6	(t)	$3'_B$, $5'_B$, 6_B'
$5'_A$		172.5	(s)		$5'_B$		172.6	(s)	
$6'_A$	1.50, s	25.2	(q)	$3'_A$, $4'_A$	$6'_B$	1.51, s	25.4	(q)	$3'_B$, $4'_B$
$1'_A'$	4.58, d (7.8)	98.5	(d)	$3'_A$, $2'_A'$	$1'_B'$	4.56, d (7.8)	98.5	(d)	$3'_B$, $2'_B'$
$2'_A'$	3.46	75.0	(d)		$2'_B'$	3.17 dd (8.1, 8.3)	75.0	(d)	
$3'_A'$	3.27	77.8	(d)		$3'_B'$	3.27	77.8	(d)	
$4'_A'$	3.29	71.6	(d)		$4'_B'$	3.29	71.6	(d)	
$5'_A'$	3.46	75.0	(d)		$5'_B'$	3.38	77.7	(d)	
6 a'_A'	4.43, dd (2.0, 11.7)	65.0	(t)	$1'_B$	6 a'_B'	3.80, dd (1.6, 11.6)	62.8	(t)	
6 b'_A'	4.11, dd (6.0, 11.7)	65.0	(t)		6 b'_B'	3.64, dd (6.0, 11.7)	62.8	(t)	
$1'_A''$	4.87, d (7.6)	105.7	(d)		$1'_B''$	4.87, d (7.6)	105.4	(d)	
$2'_A''$	3.48	75.8	(d)		$2'_B''$	3.48	75.8	(d)	
$3'_A''$	3.44	77.8	(d)		$3'_B''$	3.44	77.8	(d)	
$4'_A''$	3.43	71.4	(d)		$4'_B''$	3.43	71.4	(d)	
$5'_A''$	3.24	78.4	(d)		$5'_B''$	3.24	78.4	(d)	
6 a'_A''	3.79, dd (1.5, 11.7)	62.6	(t)		6 a'_B''	3.79, dd (1.5, 11.7)	62.6	(t)	
6 b'_A''	3.68, dd (5.4, 11.7)	62.6	(t)		6 b'_B''	3.68, dd (5.4, 11.7)	62.6	(t)	
3,5-OMe $_{A–B}$	3.85	57.3	(q)						

[a] H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) in CD_3OD, (δ in ppm, J in Hz).
[b] Multiplicities of overlapped signals are omitted.
[c] (H → C) correlations.

Table 2
Contents of compounds with relevance for nutrition in leaves and flowers of *P. orbiculare*.

	Leaves[a]	Flowers[a]
Palmitic acid	79.2 ± 11.1	54.8 ± 6.3
Linoleic acid	125.1 ± 19.1	124.4 ± 14.2
α-Linolenic acid	308.9 ± 43.5	80.5 ± 16.2
Ascorbic acid	46.8 ± 6.3	5.0 ± 0.2
β-Carotene	3.7 ± 0.3	0.7 ± 0.0
Aluminium	0.2 ± 0.2	0.4 ± 0.1
Boron	nd	nd
Barium	nd	nd
Beryllium	nd	nd
Bismuth	nd	nd
Calcium	596 ± 70.1	250.9 ± 8.5
Cadmium	nd	nd.
Cobalt	nd	nd
Chromium	nd	nd
Copper	nd	nd
Iron	1.3 ± 0.1	0.8 ± 0.1
Gallium	nd	nd
Potassium	689.3 ± 161.8	554.7 ± 2.3
Lithium	nd	nd
Magnesium	105.3 ± 20.7	78.1 ± 9.9
Sodium	38.2 ± 20.6	50.1 ± 13.0
Nickel	nd	nd
Phosphor	74.7 ± 43.1	111.2 ± 29.1
Lead	nd	nd
Selenium	nd	nd
Strontium	1.0 ± 0.3	nd
Tellurium	nd	nd
Thallium	nd	nd
Zinc	1.2 ± 0.5	0.8 ± 0.2

nd, not detected.
[a] Amounts are expressed in mg/100 g fresh weight.

The HPLC–ESIMS chromatogram of the dichloromethane extract revealed the presence of several non-UV-absorbing peaks (Fig. 3)

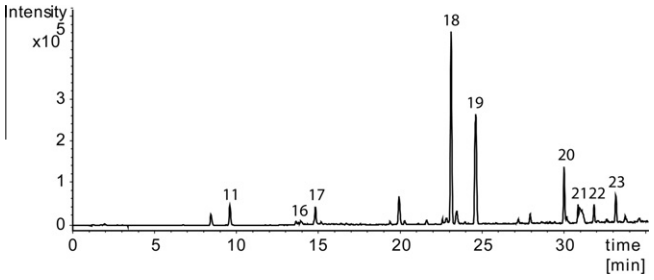

Fig. 3. HPLC–ESIMS analysis of the dichloromethane extract of dried aerial parts of *P. orbiculare* L. Base peak chromatogram, negative ion mode, *m/z* 150–1500.

which could be assigned to fatty acids and triterpenoids. Besides campesterol 3-*O*-β-glucoside (**11**), 9,12,13-trihydroxy-10,15-octadecaenoic acid (**16**), and 9,12,13-trihydroxy-10,15-octadecadienoic acid (**17**) already detected in the methanolic extract, linoleic acid (**20**), α-linolenic acid (**22**), palmitic acid (**23**), and ursolic acid (**21**) were identified from their MS data and by co-chromatography with authentic samples. Two further peaks were assigned, after small-scale purification and off-line NMR analysis, to 13-hydroxy-9,11,15-octadecatrienoic acid (**18**) and 13-hydroxy-9,11-octadecadienoic acid (**19**). Their ^{1}H- and 2D NMR data were in agreement with literature values (Waridel, Wolfender, Lachavanne, & Hostettmann, 2004).

3.2. Ascorbic acid, β-carotene, fatty acids and minerals

The fatty acid composition was analysed by GC-FID analysis after saponification of an *n*-hexane–isopropanol (9:1) extract. The major fatty acids contained in *P. orbiculare* were 18:3n3 (α-linolenic acid), 18:2n2 (linoleic acid) and 16:0 (palmitic acid) (Table 2).

The leaves possessed about 2.5 times more omega 3 (308.9 ± 43.5 mg/100 g fresh weight (FW)) than omega 6 (125.1 ± 19.1 mg/100 g FW) fatty acids, which represents a nutritionally favourable ratio (Simopoulos, 2002). Interestingly, the ratio was reversed in the flowers (10:7). A higher amount of omega 3 fatty acids is common in leafy vegetables since α-linolenic acid is an important constituent of chloroplast membrane lipids (Simopoulos, 2004).

The content of β-carotene in the leaves (3727 ± 285 μg/100 g FW) was higher than the average amounts reported for most vegetables, but only about half of that found in carotene rich plants such as parsley and spinach (USDA., 2011). The lower amount of β-carotene in the flowers (785 ± 38 μg/100 g FW) is consistent with the localization of carotenes in chloroplasts of photosynthetic tissues (Simopoulos, 2004).

Ascorbic acid has dietary significance as an antioxidant and for its role in collagen synthesis (Smirnoff, 1996). The leaves of *P. orbiculare* contained large amounts of ascorbic acid (46.8 mg/100 g FW) compared to the average content reported for green leafy vegetables (33.6 mg/100 g) (Cho et al., 2007). A portion of 100 g fresh leaves would cover around 50% of the daily recommended intake. In the flowers, the content of ascorbic acid was much lower (5.0 mg/100 g FW).

The leaves of *P. orbiculare* were found to be rich in calcium (596.0 ± 70.1 mg/100 g FW), potassium (689.3 ± 161.8 mg/100 g FW) and magnesium (105.3 ± 20.7 mg/100 g FW) (Table 2). In comparison to parsley, the leaves of *P. orbiculare* contain around six times more calcium, two times more potassium and three times more magnesium. According to the dietary reference intakes (DRI) for an adult established by the US Food and Nutrition Board of the Institute of Medicine, 100 g of leaves would meet 60% of the daily intake of calcium, 15% for potassium and 25% for magnesium (IOM., 2010). Several observational epidemiological studies and clinical trials suggest that a diet with high intake of potassium, calcium and magnesium is associated with a decrease of blood pressure and a reduced risk of cerebral infarction (Casas-Agustench, Lopez-Uriarte, Ros, Bullo, & Salas-Salvado, 2011) (Larsson, Virtamo, & Wolk, 2011). The flowers contained fewer minerals except for phosphorus, the amount of which was about twice as high as in the leaves. Heavy metals were not detected. Iron and aluminium contents were comparable to or lower than those reported for vegetables (Scancar, Stibilj, & Milacic, 2004).

3.3. Antioxidant capacity and polyphenol content

According to epidemiological studies, the consumption of fruits and vegetables that reduce oxidative stress correlates with health benefits against chronic diseases (Cho et al., 2007). The ORAC assay was used to determine the antioxidant capacity of the different plant parts. Gallic acid was selected as positive control, and its antioxidant capacity in our assay was in accordance with literature data (Yeh & Yen, 2003). As shown in Fig. 4, ORAC values of 26768 ± 113 and 19933 ± 1722 μmol of Trolox equivalents/100 g FW were determined for the leaves and flowers, respectively. This was comparable to values reported for plants commonly used as spices, such as sage (32004) or thyme (27426), but significantly higher than data for green leafy vegetables (460–8330) (Cho et al., 2007; USDA., 2010).

The concentration of total phenolic compounds was calculated as gallic acid equivalents (GAE). We determined values of 6850 ± 460 and 8240 ± 580 mg GAE/100 g FW in leaves and flowers, respectively. Cho et al. showed a strong correlation between the total phenols [mg GAE·kg^{-1} FW] and ORAC values [mmol TE·kg^{-1} FW] in 67 vegetables and 21 fruits (Cho et al., 2007). Our results match well with this correlation and indicate that phenolic components essentially contribute to the antioxidant activity of *P. orbiculare*. To obtain quantitative data on specific phenolic constituents, the content of selected compounds including chlorogenic acid (**3**), three flavonoid glycosides (**4**, **7** and **9**) and tangshenoside VII (**8**) was determined in fresh leaves and flowers by HPLC–PDA analysis (Table 3). The amount of luteolin 7-*O*-rutinoside (**7**), the most abundant polyphenol, was 68.9 ± 0.2 mg/100 g FW in flowers and 152.7 ± 1.1 mg/100 g FW in leaves.

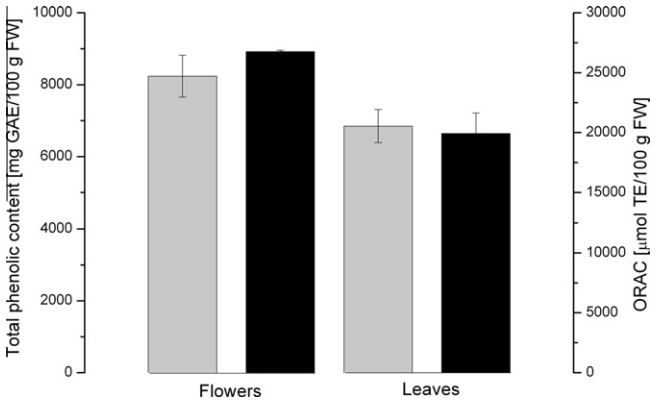

Fig. 4. Oxygen radical absorbance capacity (black) and phenolic content (grey) in flowers and leaves of *P. orbiculare*.

Table 3
Contents of selected polyphenols in leaves and flowers of *P. orbiculare*.

	Leaves[a]	Flowers[a]
Chlorogenic acid (**3**)	12.8 ± 0.5	15.6 ± 1.0
Luteolin 7-*O*-rutinoside (**7**)	152.7 ± 1.1	68.9 ± 0.2
Luteolin 7-*O*-rutinoside 4′-*O*-β-glucoside (**4**)	nd	34.7 ± 0.4
Quercetin 3-*O*-β-glucoside (**9**)	nd	10.8 ± 0.2
Tangshenoside VII (**8**)	nd	13.2 ± 0.2

nd, not detected.
[a] Amounts are expressed in mg/100 g fresh weight and are the result of triplicate injections.

3.4. Investigations of leaves and flowers of different Phyteuma species

All compounds identified in the dried aerial parts of *P.orbiculare* could also be detected in the extracts of lyophylized leaves or flowers. However, the chromatographic HPLC–PDA-ESIMS profiles of the methanol and dichloromethane extracts (Fig. 5) showed a selective distribution of some substances between both plant parts. While campesterol-3-*O*-β-glucoside (**11**), ursolic acid (**21**) as well as some flavonoids (**6**, **7**) and fatty acids (**20**, **22**, **23**) were found in flowers and in leaves, the other compounds, including the new dimeric phenylpropanoid glucoside tangshenoside VII (**8**) and the structurally unusual saponins phyteumosides A (**15**), and B (**14**) were only detected in the flowers.

Ethnobotanical information collected in our survey revealed that not only *P. orbiculare* but also other *Phyteuma* species were consumed as food plants. We therefore compared the metabolite profiles of fresh leaves and flowers of *P. orbiculare* with those of additional *Phyteuma* species growing in the Valais, including *P. spicatum*, *P. ovatum*, and *P. hemisphaericum*. To exclude climatic and environmental influences, the plants were collected at the same location and on the same day, except for *P. hemisphaericum*, which grows only at higher altitude and in siliceous soils (Holzinger, Hulber, Camenisch, & Grabherr, 2008). HPLC–PDA-ESIMS analyses revealed similarities in the metabolite profiles of most species, but substantial differences were observed for *P. hemisphaericum*. Campesterol-3-*O*-β-glucoside (**11**), ursolic acid (**21**), linoleic acid (**19**), adenosine (**1**), chlorogenic acid (**3**), *p*-hydroxybenzoic acid

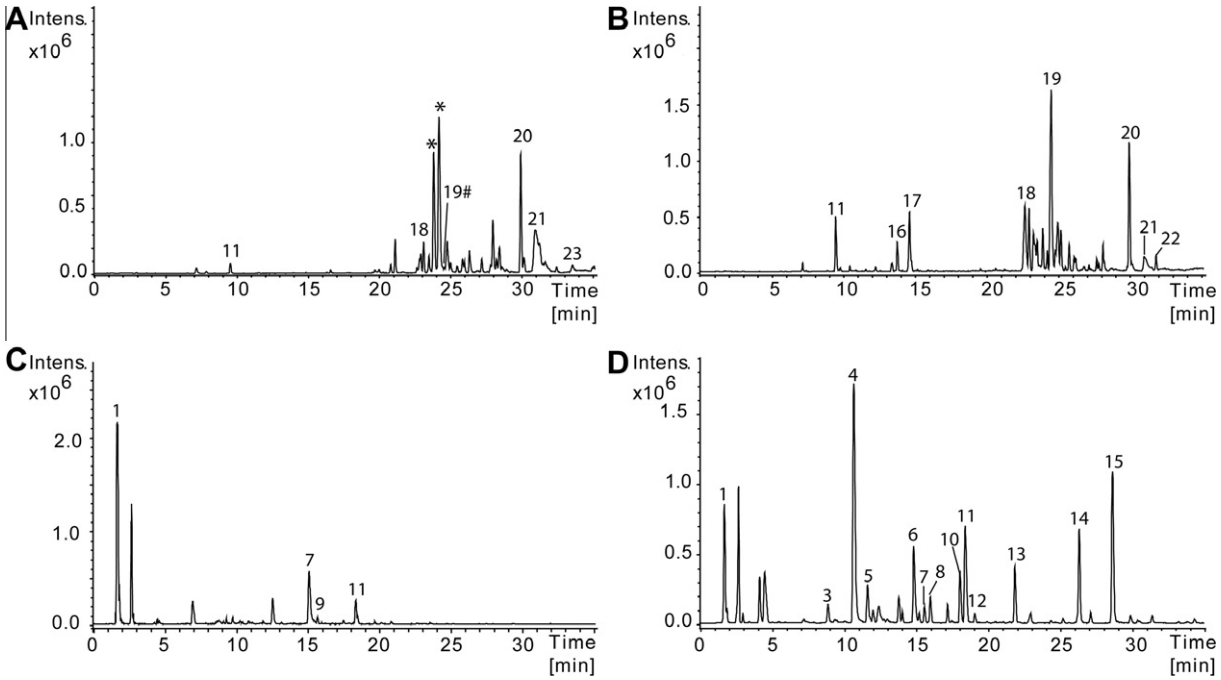

Fig. 5. Comparative analysis of the metabolite profile in freeze dried leaves and flowers of *P. orbiculare*. HPLC–ESIMS traces, base peak chromatograms, negative ion mode, *m/z* 150–1500. (A) leaves, dichloromethane extract; (B) flowers, dichloromethane extract; (C) leaves, methanol extract; (D) flowers, methanol extract. * unidentified peaks. Both compounds which showed a main peak at *m/z* 485.5 in the ESI mass spectrum were not detected in the extract of the dried aerial parts of *P. orbiculare* (Fig. 4). #Compound detected only in the extracted ion trace (*m/z* 295.5).

(**2**), most flavonoids (**4**, **7**, **9**, **10**, **12,** and **13**) and tangshenoside I (**5**) were found in all investigated species. In contrast, oxylipins (**16–19**), α-linolenic acid (**22**), palmitic acid (**23**), tangshenoside VII (**8**) and saponins (**14** and **15**) were not detected in *P. hemisphaericum* (see Supplementary Information Figs. S4–S7).

4. Conclusion

We carried out the first phytochemical analysis of *P. orbiculare*, an ancient food plant of the Valais region. The study revealed the presence of different types of secondary metabolites, including novel triterpenoid glycosides with unique structural features and a new dimeric phenylpropanoid glucoside. No compounds with reported toxicity, or substance classes with known toxicological risks were detected.

At the same time, our investigation revealed that *P. orbiculare* possesses interesting nutritive properties. The large amounts of potassium, calcium and magnesium present could help consumers to attain the recommended dietary intakes of these minerals. The leaves are particularly rich in ascorbic acid and contain, compared to the flowers, higher amounts of β-carotene, minerals and a nutritionally more favourable ratio of omega 3:omega 6 fatty acids. On the other hand, the content of total phenolic compounds is higher in the flowers and correlates with a stronger antioxidant capacity.

The chromatographic profiles revealed similar phytochemical compositions of *P. orbiculare*, *P. spicatum* and *P. ovatum*. This finding corroborates the parallel use of these three species as traditional food plants. Based on their chemical composition combined with pleasant gustatory properties, rampion species may be considered for future cultivation as food plants.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.08.018.

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Supporting Information

Comprehensive analysis of *Phyteuma orbiculare* L., a wild Alpine food plant

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Data D1. Column chromatography.

1) Open Column chromatography of the dichloromethane extract

A portion (10.3 g) of the dichloromethane extract was separated by silicagel CC (100 x 6 cm i.d.) with a gradient of *n*-hexane-ethyl acetate-methanol to give 16 fractions.

Gradient: *n*-hexane 100 % (500 mL), *n*-hexane-ethyl acetate (9:1) (1000 mL), *n*-hexane-ethyl acetate (8:2) (1000 mL), *n*-hexane-ethyl acetate (7:3) (1000 mL), *n*-hexane-ethyl acetate (1:1) (1000 mL), *n*-hexane-ethyl acetate (3:7) (1000 mL), *n*-hexane-ethyl acetate (1:9) (1000 mL), ethyl acetate 100% (1000 mL), ethyl acetate-methanol (1:1) (1000 mL), methanol 100% (2000 mL).

Volumes of the fractions: 1 (900 mL), 2 (2400 mL), 3 (500 mL), 4 (600 mL), 5 (600 mL), 6 (300 mL), 7 (600 mL), 8 (600 mL), 9 (300 mL), 10 (500 mL), 11 (400 mL), 12 (200 mL), 13 (600 mL), 14 (600 mL), 15 (700 mL), 16 (200 mL).

2) Open column chromatography of Fraction 6

Fraction 6 (418 mg) was loaded onto a silicagel column (50 x 2.5 cm i.d.) and eluted with a gradient of ethyl acetate-methanol to give 7 fractions (ca. 200 mL each).

Gradient: ethyl acetate 100 % (200 mL), ethyl acetate-methanol (50:1) (150 mL), ethyl acetate-methanol (40:1) (160 mL), ethyl acetate-methanol (30:1) (120 mL), ethyl acetate-methanol (20:1) (280 mL), ethyl acetate-methanol (10:1) (200 mL), methanol 100% (200 mL).

3) Open column chromatography of Fraction 10

100 mg of Fr. 10 (213 mg) were loaded onto a silicagel column (50 x 4 cm i.d.). The column was eluted with a gradient of *n*-hexane-ethyl acetate-methanol to give 6 fractions.

Gradient: *n*-hexane-ethyl acetate (9:1) (1000 mL), *n*-hexane-ethyl acetate (8:2) (1000 mL), *n*-hexane-ethyl acetate (7:3) (1000 mL), *n*-hexane-ethyl acetate (1:1) (1000 mL), *n*-hexane-ethyl acetate (2:8) (1000 mL), *n*-hexane-ethyl acetate (1:9) (1000 mL), ethyl acetate 100% (1600 mL), ethyl acetate-methanol (9:1) (1000 mL).

Volumes of the fractions: 1 (1000 mL), 2 (1000 mL), 3 (1000 mL), 4 (500 mL), 5 (1000 mL), 6 (2000 mL)

4) Sephadex CC of the HP20 methanol fraction

A portion (5.0 g) of HP20 methanol fraction was separated on a Sephadex LH-20 column (7 x 100 cm i.d.) with methanol to give 15 fractions.

Volumes of the fractions: 1 (400 mL), 2 (100 mL), 3 (30 mL), 4 (50 mL), 5 (40 mL), 6 (70 mL), 7 (70 mL), 8 (90 mL), 9 (50 mL), 10 (70 mL), 11 (50 mL), 12 (50 mL), 13 (60 mL), 14 (110 mL), 15 (200 mL).

Data D2. Chromatograms of the separations by semi preparative HPLC

SunFire C_{18} column 5 μm, 150 x 10.0 mm I.D. (Waters, Milford, MA, USA) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to a DAD detector (λ=254 nm). Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeOH, flow rate: 4 mL/min, column temperature: 25 °C, injected sample were dissolved in the respective starting HPLC solvent systems.

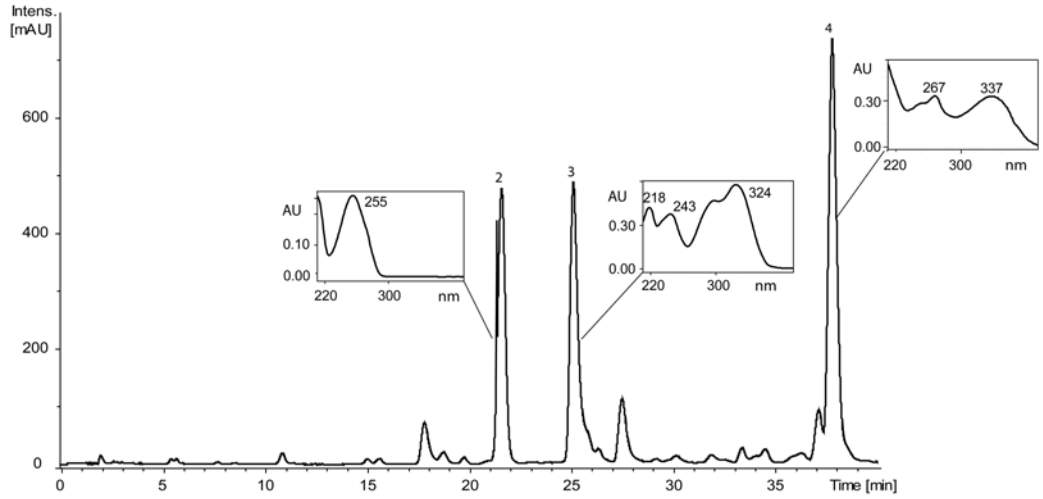


Figure 1. Semi-preparative HPLC of Fr. 11. Gradient: 5% to 25% B in 25 min, then to 40% B in 25 min.

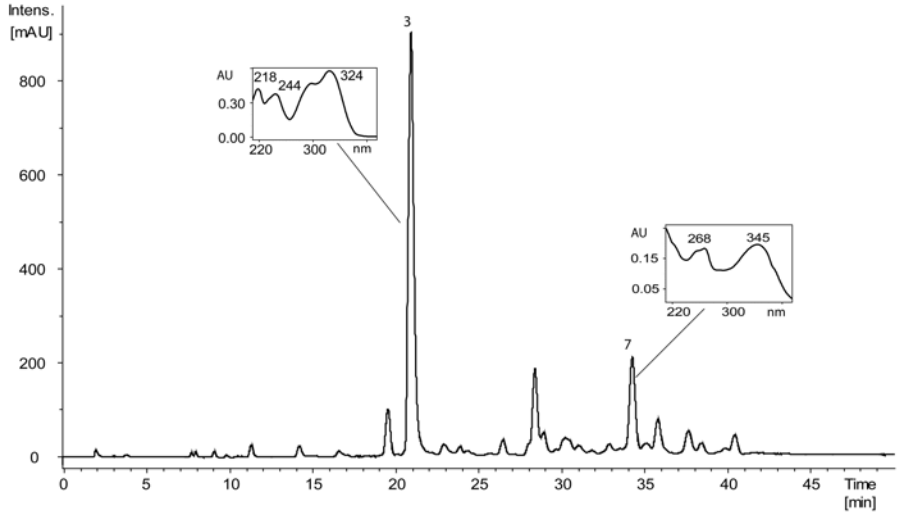


Figure 2. Semi-preparative HPLC of Fr. 12. Gradient: 5% to 50% B in 40 min, then to 60% B in 10 min.

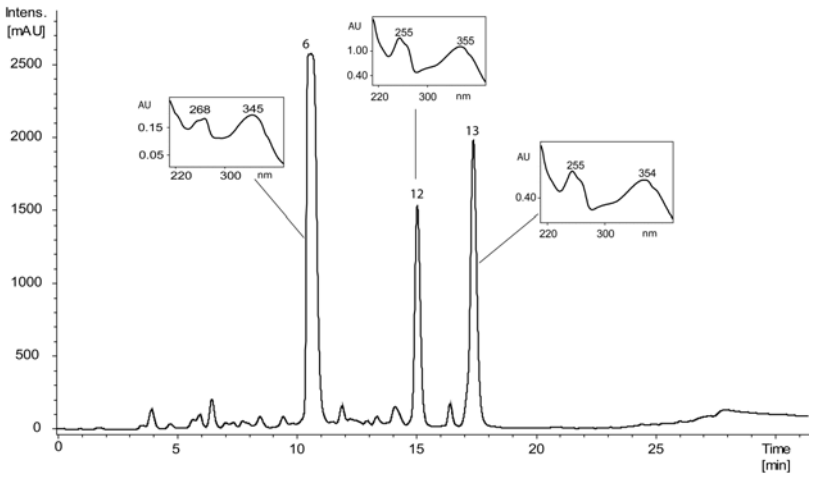


Figure 3. Semi-preparative HPLC of Fr. 14. Gradient: 15% to 60% B in 30 min, then to 100% B in 10 min.

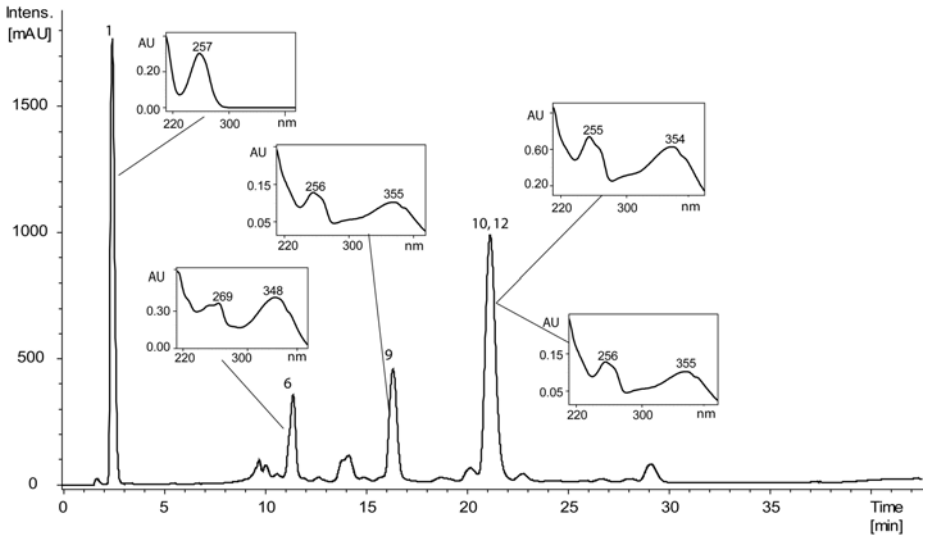


Figure 4. Semi-preparative HPLC of Fr. 15. Gradient: 25% to 29% in 40 min.

Data D3. Physical and spectroscopic data of the isolated compounds

Adenosine (**1**). White powder. ESI-MS: *m/z* 266 $[M-H]^-$. UV (MeOH) λ_{max} nm: 255. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.57 (1H, *dd*, *J* = 3.0, *J* = 12.1, H_a-6'), 3.68 (1H, *dd*, *J* = 3.0, *J* = 12.1, H_b-6'), 3.98 (1H, *d*, *J* = 2.8, H-4'), 4.17 (1H, *dd*, *J* = 3.1, *J* = 3.5, H-3'), 4.61 (1H, *dd*, *J* = 5.2, *J* = 5.4, H-2'), 5.89 (1H, *d*, *J* = 5.9, H-1'), 7.22 (1H, *s*, H-NH2), 8.14 (1H, *s*, H-8), 8.32 (1H, *s*, H-2). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 61.9 (C-5'), 62.0 (C-6'), 71.1 (C-3'), 74.0 (C-2'), 86.2 (C-4'), 88.5 (C-1'), 119.8 (C-5), 140.2 (C-8), 149.2 (C-4), 156.6 (C-6).

Chlorogenic acid (**3**). White powder. ESI-MS: *m/z* 353 $[M-H]^-$. UV (MeOH) λ_{max} nm: 218, 244, 324. ^{1}H-NMR (DMSO-d_6, 500 MHz): Caffeoyl moiety: δ 6.17 (1H, *d*, *J* = 15.9, H-8), 6.76 (1H, *d*, *J* = 8.1, H-5), 6.94 (1H, *dd*, *J* = 1.3, *J* = 8.1, H-6), 7.02 (1H, *d*, *J* = 1.3, H-2), 7.41 (1H, *d*, *J* = 15.9, H-7); quinic acid moiety: δ 1.79 (1H, *dd*, *J* = 6.2, *J* = 13.6, H_b-2), 1.95 (2H, *d*, *J* = 5.7, H-6), 2.01 (1H, *dd*, *J* = 2.5, *J* = 13.6, H_a-2), 3.58 (1H, *dd*, *J* = 2.5, *J* = 7.5, H-4), 3.96 (1H, *m*, H-5), 5.08 (1H, *dd*, *J* = 6.6, *J* = 13.0, H-3). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra), caffeoyl moiety: 114.6 (C-2), 114.8 (C-8), 116.2 (C-5), 121.9 (C-6), 126.2 (C-1), 145.5 (C-7), 146.1 (C-3), 148.3 (C-4), 167.5 (C-9); quinic acid moiety : δ 37.1 (C-6), 37.3 (C-2), 69.1 (C-3), 71.2 (C-4), 71.2 (C-5), 71.5 (C-1).

Luteolin 7-*O*-β-rutinoside 4'-*O*-*β*-glucoside (**4**). Yellow powder. ESI-MS: *m/z* 755 $[M-H]^-$. UV (MeOH) λ_{max} nm: 267, 337. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.08 (3H, *d*, *J* = 6.2, H_{Rha}-6), 3.13-3.24 (3H, *m*, H_{Glc1}-4, H_{Glc2}-4, H_{Rha}-4), 3.26-3.54 (9H, *m*, H_{Glc1}-2, H_{Glc1}-3, H_{Glc1a}-6, H_{Rha}-3, H_{Rha}-5, H_{Glc2}-2, H_{Glc2}-3, H_{Glc2}-5, H_{Glc2a}-6), 3.64 (2H, *m*, H_{Rha}-2, H_{Glc1}-5), 3.75 (1H, *dd*, *J* = 1.5, *J* = 11.7, H_{Glc2b}-6), 3.86 (1H, *dd*, *J* = 1.0, *J* = 10.9, H_{Glc1b}-6), 4.57 (1H, br *s*, H_{Rha}-1), 4.88 (1H, *d*, *J* = 7.1, H_{Glc2}-1), 5.07 (1H, *d*, *J* = 7.5, H_{Glc1}-1), 6.46 (1H, br *s*, H-6), 6.78 (1H, br *s*, H-8), 6.90 (1H, *d*, *J* = 8.3, H-2'), 7.51 (2H, *dd*, *J* = 1.8, *J* = 8.3, H-1', H-5'), 7.92 (1H, *d*, *J* = 1.8, H-2'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 17.4 (C_{Rha}-6), 51.3 (3'OMe), 60.5 (C_{Glc2}-6), 65.8 (C_{Glc1}-6), 67.9 (C_{Rha}-5), 69.5 (C_{Glc1}-4, C_{Glc2}-4), 69.9 (C_{Rha}-2), 70.4 (C_{Rha}-3), 71.8 (C_{Rha}-4), 72.8 (C_{Glc1}-2, C_{Glc2}-2), 75.3 (C_{Glc1}-5), 75.6 (C_{Glc1}-3), 75.8 (C_{Glc2}-3), 76.9 (C_{Glc2}-5), 94.3 (C-8), 99.2 (C-6), 99.5 (C_{Glc1}-1), 100.0 (C_{Rha}-1), 101.0 (C_{Glc2}-1), 103.9 (C-3), 105.3 (C-4a), 113.4 (C-2'), 115.9 (C-5'), 118.1 (C-6'), 123.1 (C-1'), 146.8 (C-3'), 163.0 (C-7), 181.2 (C-4).

Luteolin 6-*C*-β-glucoside (**6**). Yellow powder. ESI-MS: *m/z* 447 $[M-H]^-$. UV (MeOH) λ_{max} nm: 257, 268, 348. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.19 (1H, *m*, H_{Glc}-2), 3.23 (2H, *m*, H_{Glc}-3, H_{Glc}-5), 3.45 (1H, *dd*, *J* = 5.1, *J* = 11.5, H_{Glca}-6), 3.69 (1H, *d*, *J* = 10.5, H_{Glcb}-6), 4.05 (1H, *dd*, *J* = 8.9, *J* = 9.4, H_{Glc}-4), 4.62 (1H, *d*, *J* = 9.8, H_{Glc}-1), 6.47 (1H, *s*, H-8), 6.62 (1H, *s*, H-3), 6.89 (1H, *d*, *J* = 8.0, H-5'), 7.40 (2H, *dd*, *J* = 2.1, *J* = 9.1, H-2', H-6'), 13.54 (OH-5). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra) : δ 61.2 (C_{Glc}-6), 70.1 (C_{Glc}-4), 70.4 (C_{Glc}-2), 73.0 (C_{Glc}-1), 78.7 (C_{Glc}-3), 81.2 (C_{Glc}-5), 93.5 (C-8), 102.4 (C-3), 109.1 (C-6), 113.0 (C-2'), 115.8 (C-5'), 118.7 (C-6'), 121.5 (C-1'), 146.2 (C-3'), 150.4 (C-4'), 160.9 (C-5), 164.3 (C-7), 182.1 (C-4).

Luteolin 7-*O*-β-rutinoside (**7**). Yellow powder. ESI-MS: *m/z* 593 $[M-H]^-$. UV (MeOH) λ_{max} nm: 268, 345; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.08 (3H, *d*, *J* = 6.3, H_{Rha}-6), 3.18 (2H, *m*, H_{Glc}-4, H_{Rha}-4), 3.26-3.34 (2H, *m*, H_{Glc}-2, H_{Glc}-3), 3.46 (3H, *m*, H_{Rha}-3, H_{Rha}-5, H_{Glca}-6), 3.59 (1H, *dd*, *J* = 8.1, *J* = 7.7, H_{Glc}-5), 3.67 (1H, *dd*, *J* = 1.3, *J* = 3.3, H_{Rha}-2), 3.86 (1H, br *dd*, *J* = 11.1, *J* = 11.8, H_{Glcb}-6), 4.56 (1H, br *s*, H_{Rha}-1), 5.05 (1H, *d*, *J* = 7.5, H_{Glc}-1), 6.45 (1H, *d*, *J* = 1.8, H-6), 6.68 (1H, *s*, H-3), 6.72 (H, *d*, *J* = 1.8, H-8), 6.89 (1H, *d*, *J* = 8.0, H-5'), 7.40 (1H, *d*, *J* = 1.7, H-2'), 7.42 (1H, *dd*, *J* = 8.2, *J* = 1.7, H-6'). ^{13}C-NMR (DMSO-d_6,

extracted from 2D-HMBC and HSQC spectra): δ 18.2 (C_{Rha}-6), 66.5 (C_{Glc}-6), 68.7 (C_{Rha}-5), 70.0 (C_{Rha}-4), 70.7 (C_{Rha}-2), 71.3 (C_{Rha}-3), 72.5 (C_{Glc}-4), 73.5 (C_{Glc}-2), 76.1 (C_{Glc}-5), 76.8 (C_{Glc}-3), 95.4 (C-8), 99.8 (C-6), 100.4 (C_{Glc}-1), 100.9 (C_{Rha}-1), 103.7 (C-3), 113.5 (C-2'), 116.4 (C-5'), 119.6 (C-6').

Quercetin 3-*O*-β-glucoside (**9**). Yellow powder; ESI-MS: *m/z* 463 $[M-H]^-$; UV (MeOH) λ_{max} nm: 256, 355; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.11(2H, *m*, H_{Glc}-4, H_{Glc}-5), 3.24 (2H, *m*, H_{Glc}-2, H_{Glc}-3), 3.35 (1H, *dd*, *J* = 5.0, *J* = 11.8, H_{Glcb}-6), 3.58 (1H, *bd*, *J* = 11.4, H_{Glca}-6), 5.43 (1H, *d*, *J* = 7.3, H_{Glc}-1), 6.19 (1H, *d*, *J* = 1.5, H-6), 6.40 (1H, *d*, *J* = 1.5, H-8), 6.85 (1H, *d*, *J* = 8.2, H-5'), 7.57 (2H, *m*, H-2', H-6'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 60.8 (C_{Glc}-6), 69.8 (C_{Glc}-4), 73.9 (C_{Glc}-2), 76.3 (C_{Glc}-3), 77.2 (C_{Glc}-5), 93.4 (C-8), 98.5 (C-6), 100.9 (C_{Glc}-1), 104.6 (C-10), 115.1 (C-2'), 116.0 (C-5'), 121.1 (C-1'), 121.7 (C-6'), 145.1 (C-3'), 148.6 (C-4'), 149.4 (C-9), 156.7 (C-2), 161.7 (C-5), 164.8 (C-7).

Quercetin 3-*O*-(6$_{Glc}$-*O*-malonyl)-β-glucoside (**10**). Yellow powder. ESI-MS: *m/z* 549 $[M-H]^-$. UV (MeOH) λ_{max} nm: 256, 355. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.96 (2H, br *s*, H_{mal}-2), 3.21 (4H, *m*, H_{Glc}-2, H_{Glc}-3, H_{Glc}-4, H_{Glc}-5), 3.99 (1H, *dd*, *J* = 5.3, *J* = 11.8, H_{Glcb}-6), 4.16 (1H, *dd*, *J* = 1.4, *J* = 11.8, H_{Glca}-6), 5.33 (1H, *d*, *J* = 7.2, H_{Glc}-1), 6.20 (1H, br *s*, H-6), 6.40 (1H, br *s*, H-8), 6.84 (1H, *d*, *J* = 8.3, H-5'), 7.45 (1H, *dd*, *J* = 1.8, *J* = 8.3, H-6'), 7.51 (1H, *d*, *J* = 1.8, H-2'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra) : δ 43.1 (C_{mal}-2), 62.5 (C_{Glc}-6), 69.1 (C_{Glc}-4), 73.5 (C_{Glc}-2, C_{Glc}-5), 75.9 (C_{Glc}-3), 93.3 (C-8), 98.4 (C-6), 101.0 (C_{Glc}-1), 103.7 (C-10), 114.8 (C-2'), 115.9 (C-5'), 120.8 (C-1'), 144.4 (C-3'), 148.3 (C-4'), 156.3 (C-9), 156.6 (C-2), 160.9 (C-5), 164.6 (C-7), 167.8 (C_{mal}-1), 168.7 (C_{mal}-3).

Campesterol 3-*O*-β-glucoside (**11**). White powder. ESI-MS: *m/z* 607 $[M+HCOO^-]^-$. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 0.72 (3H, *s*, H-18), 0.90 (3H, *d*, *J* = 3.8, H-28), 0.91 (3H, *d*, *J* = 7.0, H-27), 0.91 (3H, *d*, *J* = 7.0, H-26), 0.96 (1H, *m*, H-9), 0.98 (3H, *s*, H-19), 1.00 (1H, *m*, H-14), 1.03 (3H, *d*, *J* = 6.5, H-21), 1.04-1.11 (3H, *m*, H_a-1, H_a-16, H-24), 1.15 (1H, *m*, H_a-22), 1.19-1.20 (2H, *m*, H_a-12, H-17), 1.32-1.35 (3H, *m*, H_b-15, H_a-23 H_b-23), 1.40 (1H, *m*, H-20), 1.45 (1H, *m*, H-8), 1.50 (1H, *m*, H-11), 1.60 (1H, *m*, H_b-16), 1.73 (1H, *m*, H-25), 1.78 (2H, *m*, H_a-2, H_b-1), 1.90 (1H, *m*, H_a-15), 1.95 (1H, *m*, H_a-7), 1.99 (1H, *m*, H_b-7), 2.00 (1H, *m*, H_b-12), 2.17 (1H, *m*, H_b-2), 2.50 (1H, *m*, H_a-4), 2.74 (1H, *m*, H_b-4), 4.25 (1H, *m*, H-3), 5.39 (1H, *m*, H-6). ^{13}C-NMR (pyridine-d_5, extracted from 2D-HMBC and HSQC spectra) : 12.3 (C-18), 12.5 (C-28), 19.3 (C-21), 19.5 (C-19), 19.8 (C-26), 20.3 (C-27), 21.6 (C-11), 23.4 (C-23), 24.5 (H-16), 28.5 (C-15), 29.8 (C-25), 30.6 (C-2), 32.2 (C-8), 32.5 (C-7), 34.5 (C-22), 36.7 (C-20), 37.2 (C-10), 37.8 (C-1), 39.6 (C-4), 40.2 (C-12), 42.8 (C-13), 46.3 (C-24), 49.9 (C-9), 56.5 (C-17), 57.1 (C-14), 78.9 (C-3), 122.2 (C-6), 141.2 (C-5).

Isorhamnetin 3-*O*-β-glucoside (**12**). Yellow powder; ESI-MS: *m/z* 477 $[M-H]^-$; UV (MeOH) λ_{max} nm: 255, 354; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.20 (4H, *m*, H_{Glc}-2, H_{Glc}-3, H_{Glc}-4, H_{Glc}-5), 3.40 (1H, *dd*, *J* = 4.6, *J* = 11.7, H_{Glcb}-6), 3.60 (1H, *dd*, *J* = 11.7, H_{Glca}-6), 3.84 (3H, *s*, 3'-OMe), 5.53 (1H, *d*, *J* = 7.2, H_{Glc}-1), 6.20 (1H, br *d*, H-6), 6.43 (1H, br *d*, H-8), 6.90 (1H, *d*, *J* = 8.3, H-5'), 7.51 (1H, *dd*, *J* = 1.8, *J* = 8.3, H-6'), 7.92 (1H, *d*, *J* = 1.8, H-2'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra) : δ 55.6 (3'-OMe), 60.4 (C_{Glc}-6), 69.7 (C_{Glc}-4), 74.0 (C_{Glc}-2), 76.2 (C_{Glc}-3), 77.1 (C_{Glc}-5), 93.6 (C-8), 98.7 (C-6), 100.7 (C_{Glc}-1), 104.1 (C-10), 113.3 (C-2'), 115.0 (C-5'), 121.4 (C-1'), 121.8 (C-6'), 147.1 (C-4'), 149.2 (C-3'), 156.9 (C-2), 157.6 (C-9), 161.5 (C-5), 164.9 (C-7).

Isorhamnetin 3-*O*-(6$_{Glc}$-*O*-malonyl)-β-glucoside (**13**). Yellow powder. ESI-MS: *m/z* 563 [M-H]$^-$. UV (MeOH) λ_{max} nm: 256, 355. ^{1}H-NMR (DMSO-d$_6$, 500 MHz): δ 3.00 (2H, br *s*, H$_{mal}$-2), 3.24 (4H, *m,* H$_{Glc}$-2, H$_{Glc}$-3, H$_{Glc}$-4, H$_{Glc}$-5), 3.85 (3H, *s*, 3'-OMe), 4.10 (2H, *m*, H$_{Glca}$-6, H$_{Glcb}$-6), 5.41 (1H, *d*, *J* = 6.7, H$_{Glc}$-1), 6.22 (1H, br *s*, H-6), 6.46 (1H, br *s*, H-8), 6.92 (1H, *d*, *J* = 8.5, H-5'), 7.54 (1H, *dd*, *J* = 1.6, *J* = 8.5, H-6'), 7.81 (1H, br *s*, H-2'). ^{13}C-NMR (DMSO-d$_6$, extracted from 2D-HMBC and HSQC spectra) : δ 41.9 (C$_{mal}$-2), 55.5 (3'-OMe), 63.0 (C$_{Glc}$-6), 69.4 (C$_{Glc}$-4), 73.6 (C$_{Glc}$-2), 74.0 (C$_{Glc}$-5), 76.0 (C$_{Glc}$-3), 93.5 (C-8), 98.5 (C-6), 101.0 (C$_{Glc}$-1), 103.4 (C-10), 113.1 (C-2'), 115.0 (C-5'), 120.2 (C-1'), 122.0 (C-6'), 146.2 (C-4'), 149.5 (C-3'), 156.0 (C-9), 156.3 (C-2), 161.3 (C-5), 164.3 (C-7), 167.0 (C$_{mal}$-1), 168.8 (C$_{mal}$-3).

9,12,13-Trihydroxy-10,15-octadecaenoic acid (**16**). White powder. ESI-MS: *m/z* 329 [M-H]$^-$. ^{1}H-NMR (CD$_3$OD, 500 MHz): δ 0.75 (3H, *t*, *J* = 7.4, H-18), 1.16-1.39 (18H, *m*, H-3, H-4, H-5, H-6, H-7, H-8, H-14, H-15, H-16), 2.27 (2H, *t*, *J* = 7.3, H-2), 3.44 (1H, *m*, H-13), 3.92 (1H, *t*, *J* = 6.0, *J* = 6.0, H-12), 4.07 (1H, *dd*, *J* = 6.0, *J* = 12.3, H-9), 5.77 (2H, *m*, H-11, H-16). ^{13}C-NMR (CD$_3$OD, extracted from 2D-HMBC and HSQC spectra) : 13.2 (C-18), 22.5 (C-17), 25.3-32.6 (C-3,4,5,6,7,14,15), 35.6 (C-2), 37.3 (C-8), 71.9 (C-9), 74.7 (C-13), 75.3 (C-12), 130.0 (C-16), 135.5 (C-11), 178.8 (C-1).

9,12,13- Trihydroxy-10,15-octadecadienoic acid (**17**). White powder. ESI-MS: *m/z* 327 [M-H]$^-$.. ^{1}H-NMR (CD$_3$OD, 500 MHz): δ 0.97 (3H, *t*, *J* = 7.4, H-18), 1.23-1.78 (12H, *m*, H-3, H-4, H-5, H-6, H-7, H-8), 2.07 (3H, *m*, H-17), 2.22 (3H, *t*, *J* = 7.4, H-2), 3.47 (1H, *m*, H-13), 3.96 (1H, *t*, *J* = 5.6, H-12), 4.06 (1H, *q*, *J* = 6.5, *J* = 11.8, H-9), 5.46 (2H, *m*, H-15, H-16), 5.74 (2H, *m*, H-10, H-11). ^{13}C-NMR (CD$_3$OD, extracted from 2D-HMBC and HSQC spectra) : 13.2 (C-18), 20.2 (C-17), 25.0 (C-6), 25.5 (C-3), 29.0 (C-4,5), 30.5 (C-14), 35.9 (C-2), 37.0 (C-8), 71.6 (C-9), 74.4 (C-12, C-13), 125.0 (C-15), 129.8 (C-10), 133.0 (C-16), 135.1 (C-11), 179.7 (C-1).

13-hydroxy-9,11,15-octadecatrienoic acid (**18**). Yellow powder. ESI-MS: *m/z* 293 [M-H]$^-$. UV (MeOH) λ_{max} nm: 234; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 0.97 (3H, *t*, *J* = 7.6, H-18), 1.32 (6H, *m*, H-4, H-5, H-6), 1.38 (2H, *m*, H-7), 1.63 (2H, *m*, H-3), 2.07 (2H, *qd*, *J* = 7.3, *J* = 7.5, H-17), 2.18 (2H, *m*, H-8), 2.35 (4H, *t*, *J* = 7.6, H-2, H-14), 4.22 (1H, *dd*, *J* = 6.4, *J* = 6.1, H-13), 5.37 (1H, *J* = 7.5, *J* = 18.0, H-15), 5.43 (1H, *dd*, *J* = 7.8, *J* = 18.0, H-9), 5.57 (1H, *dd*, *J* = 7.3, *J* = 17.9, H-16), 5.69 (1H, *dd*, *J* = 15.1, *J* =6.3, H-12), 5.97 (1H, *t*, *J* = 10.9, H-10), 6.51 (1H, *dd*, *J* = 11.2, *J* = 14.9, H-11). ^{13}C-NMR (CDCl$_3$, extracted from 2D-HMBC and HSQC spectra) : δ 13.7 (C-18), 20.5 (C-17), 24.6 (C-3), 27.7 (C-8), 29.0 (C-4, C-5, C-6), 29.7 (C-7), 33.7 (C-2), 34.8 (C-14), 71.8 (C-13), 123.5 (C-15), 125.6 (C-11), 127.2 (C-10), 132.2 (C-9), 134.5 (C-12, C-16), 178.3 (C-1).

Table 1. ^{1}H- and ^{13}C-NMR Data of tangshenosides I (**5**) and VII (**8**) in CD_3OD[a]

	Tangshenoside VII (**8**)			Tangshenoside I (**5**)[b]		
position	$\delta_H$mult.[a]	δ_C mult.		$\delta_H$mult.[b]	δ_C mult.	
1_A		134.6	(s)		133.6	(s)
2_A-6_A	6.75, *s*	105.4	(d)	6.71, *s*	105.1	(d)
3_A -5_A		154.4	(d)		153.3	(d)
4_A		136.3	(s)		135.3	(s)
α_A	4.73, *d* (6.1)	66.3	(t)	4.69, *d* (6.1)	65.3	(t)
β_A	6.26, *ddd* (1.0, 6.1, 7.4)	124.5	(d)	6.24, *ddd*	123.6	(d)
γ_A	6.60	135.2	(d)	6.58	134.2	(d)
1_A’		172.6	(s)		170	(s)
2_A’	2.76-2.84	44.8	(t)	2.86	44.1	(t)
3_A’		76.1	(s)		76.1	(s)
4_A’	2.87-2.95	44.9	(t)	2.86	44.1	(t)
5_A’		172.5	(s)		171.1	(s)
6_A’	1.50, *s*	25.2	(q)	1.48	24.4	(q)
1_A’’	4.58, *d* (7.8)	98.5	(d)	4.58, *d* (7.8)	98.5	(d)
2_A’’	3.46	75.0	(d)	3.46	75.0	(d)
3_A’’	3.27	77.8	(d)	3.27	77.8	(d)
4_A’’	3.29	71.6	(d)	3.29	71.6	(d)
5_A’’	3.46	75.0	(d)	3.46	75.0	(d)
6 a_A’’	4.43, *dd* (2.0, 11.7)	65.0	(t)	4.43, *dd* (2.0, 11.7)	65.1	(t)
6 b_A’’	4.11, *dd* (6.0, 11.7)	65.0	(t)	4.11, *dd* (6.0, 11.7)	65.1	(t)
1_A’’’	4.87, *d* (7.6)	105.7	(d)	4.87, *d* (7.5)	105.5	(d)
2_A’’’	3.48	75.8	(d)		75.8	(d)
3_A’’’	3.44	77.8	(d)		77.8	(d)
4_A’’’	3.43	71.4	(d)		71.4	(d)
5_A’’’	3.24	78.4	(d)		78.4	(d)
6 a_A’’’	3.79, *dd* (1.5, 11.7)	62.6	(t)	3.79, *dd* (11.6)	62.6	(t)
6 b_A’’’	3.68, *dd* (5.4, 11.7)	62.6	(t)	3.68, *dd* (5.4, 11.8)	62.6	(t)
3,5-OMe $_{A\text{-}B}$	3.85	57.3	(q)		57.3	(q)
1_B		134.6	(s)			
2_B-6_B	6.71	105.4	(d)			
3_B -5_B		154.4	(d)			
4_B		136.2	(s)			
α_B	4.73	66.3	(t)			
β_B	6.29, *ddd* (1.0, 6.1, 7.4)	124.4	(d)			
γ_B	6.63	135.2	(d)			
1_B’		172.6	(s)			
2_B’	2.76-2.84	44.5	(t)			
3_B’		76.1	(s)			
4_B’	2.87-2.95	44.6	(t)			
5_B’		172.6	(s)			
6_B’	1.51, *s*	25.4	(q)			
1_B’’	4.56, *d* (7.8)	98.5	(d)			
2_B’’	3.17 *dd* (8.1, 8.3)	75.0	(d)			
3_B’’	3.27	77.8	(d)			
4_B’’	3.29	71.6	(d)			
5_B’’	3.38	77.7	(d)			
6 a_B’’	3.80, *dd* (1.6, 11.6)	62.8	(t)			
6 b_B’’	3.64, *dd* (6.0, 11.7)	62.8	(t)			
1_B’’’	4.87, *d* (7.6)	105.4	(d)			
2_B’’’	3.48	75.8	(d)			
3_B’’’	3.44	77.8	(d)			
4_B’’’	3.43	71.4	(d)			
5_B’’’	3.24	78.4	(d)			
6 a_B’’’	3.79, *dd* (1.5, 11.7)	62.6	(t)			
6 b_B’’’	3.68, *dd* (5.4, 11.7)	62.6	(t)			

a ^{1}H-NMR data at 500 MHz.
[b] ^{13}C-NMR data 0f **5** extracted from 2D-HMBC and HSQC spectra, (δ in ppm, J in Hz). [b]Multiplicities of overlapped signals are omitted.

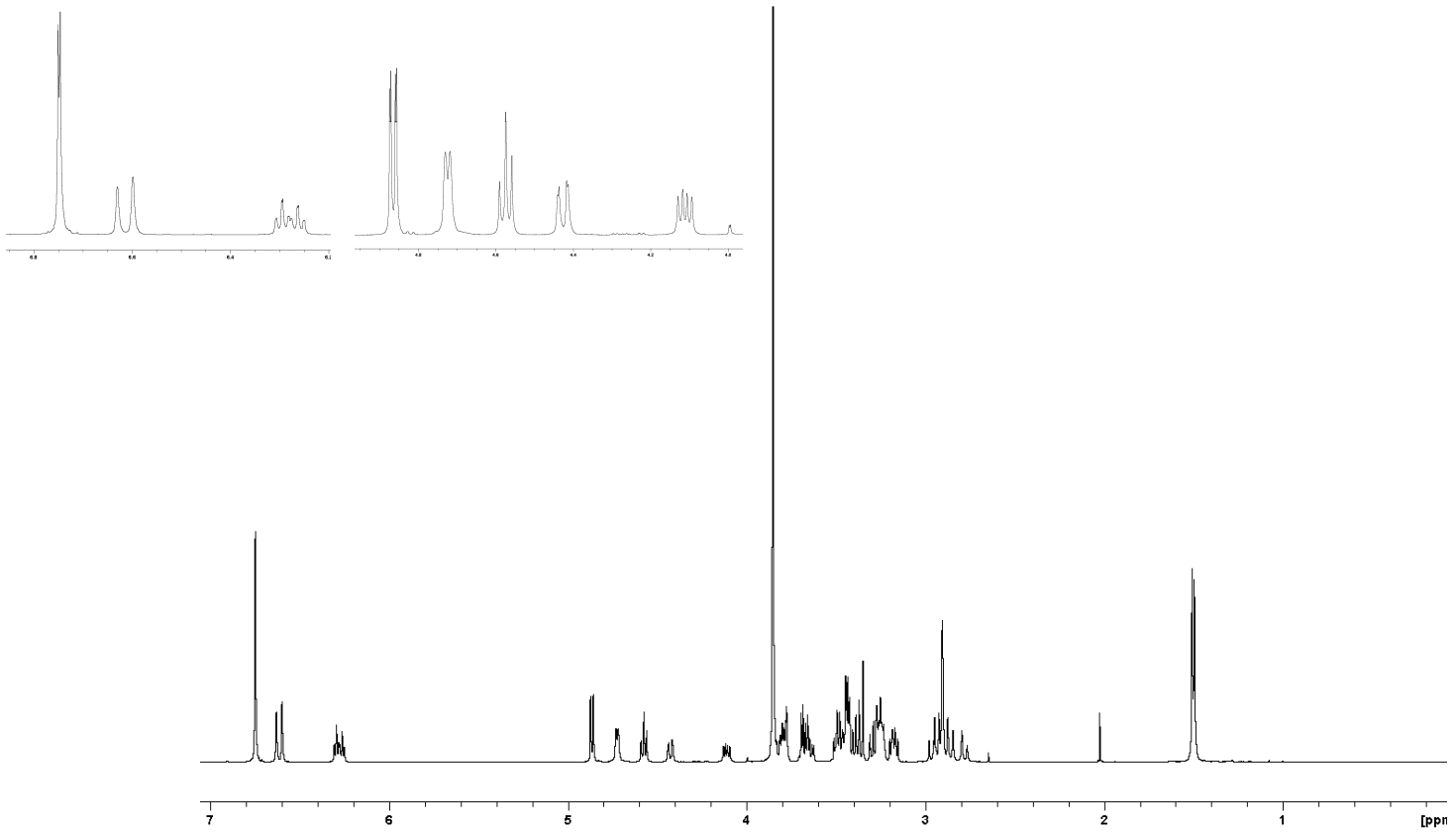


Figure S1. ^{1}H-NMR spectrum of tangshenoside VII (**8**) in CD_3OD.

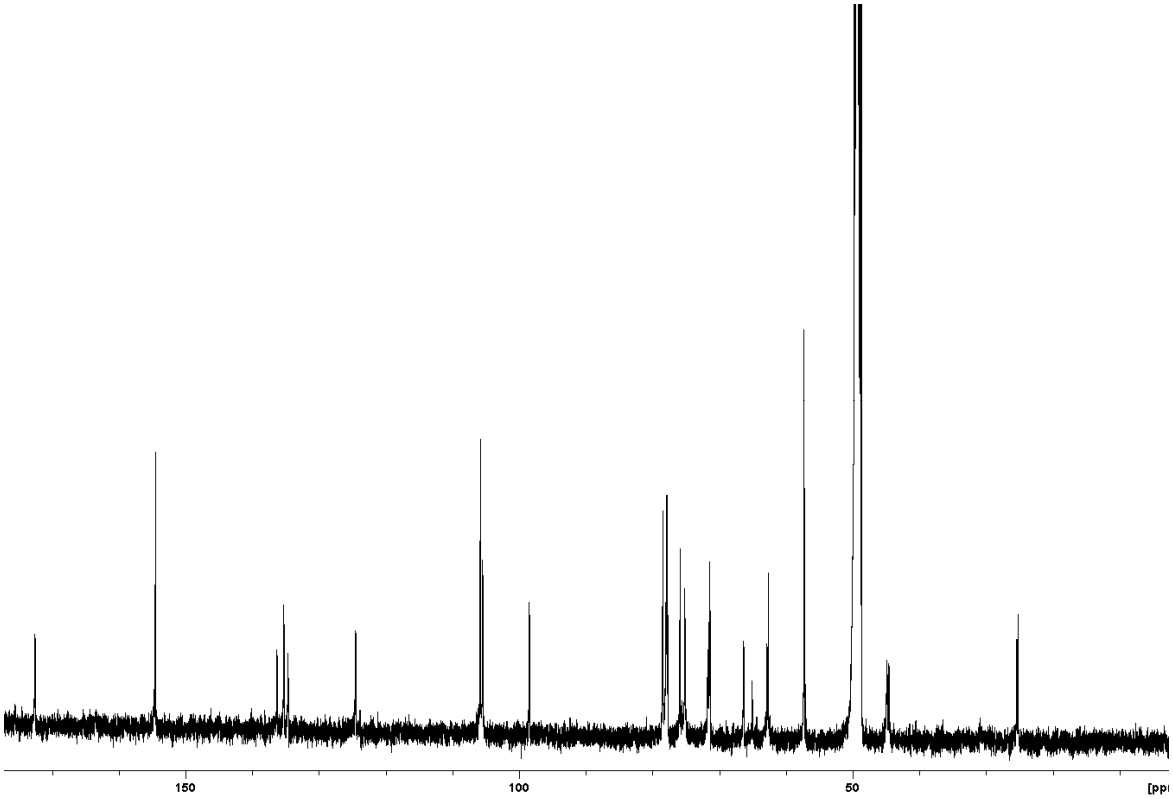


Figure S2. ^{13}C-NMR spectrum of tangshenoside VII (**8**) in CD_3OD.

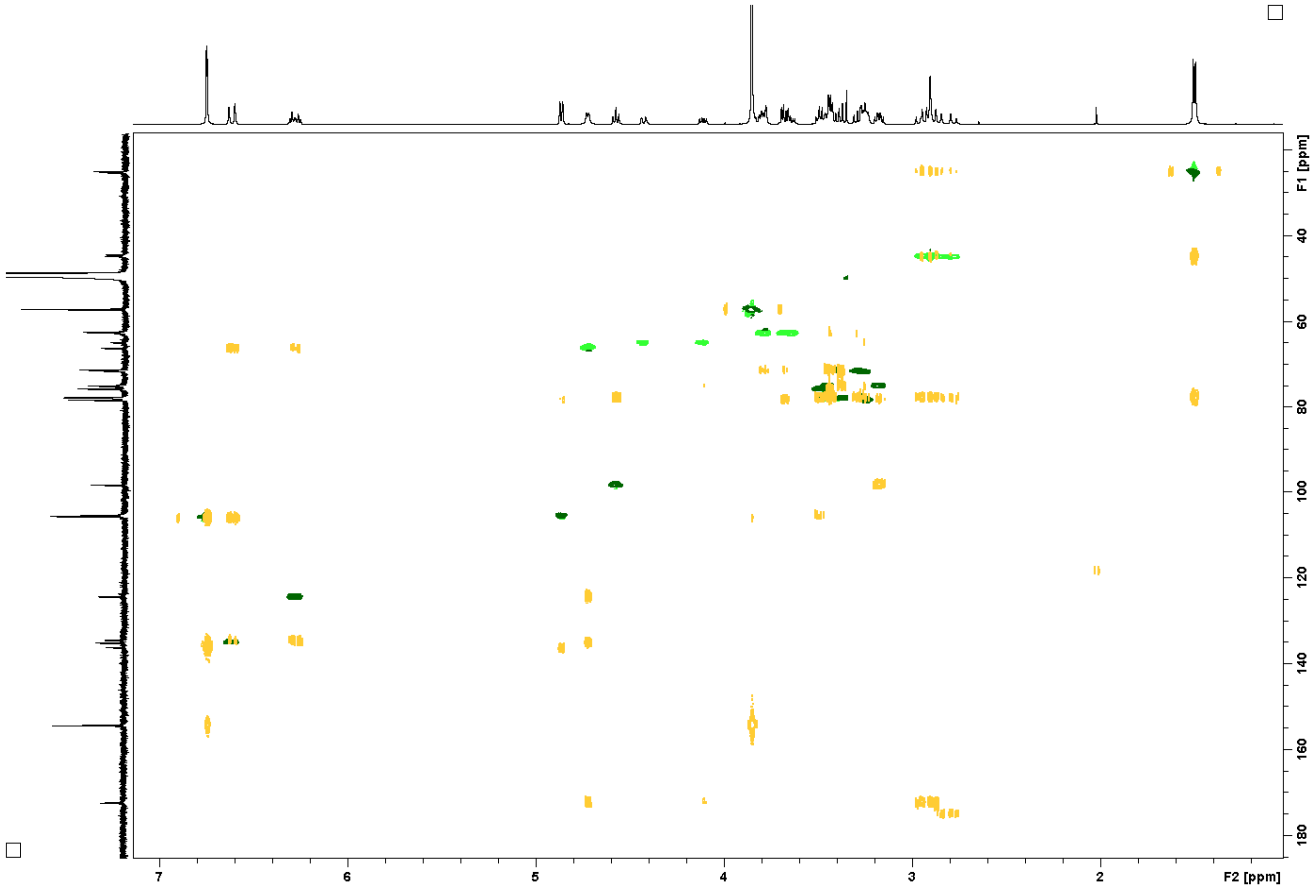
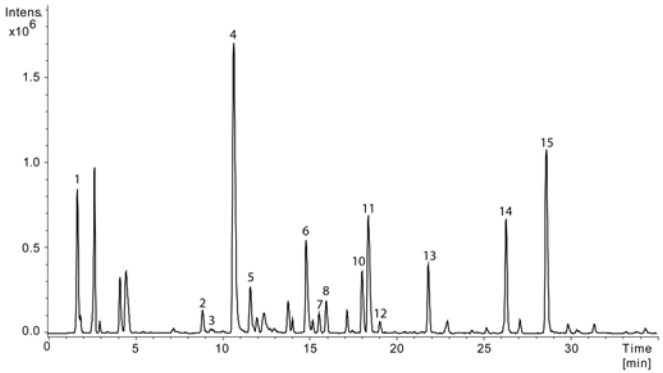
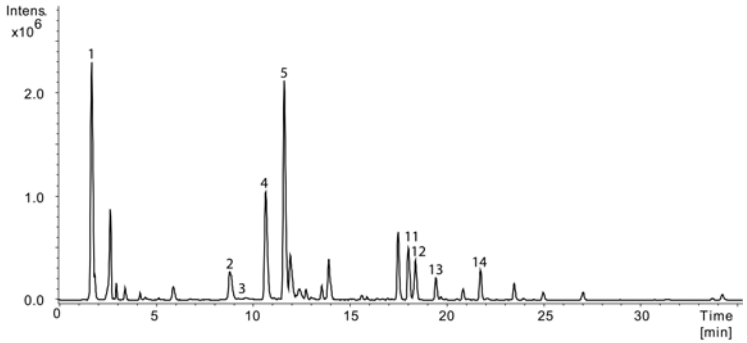


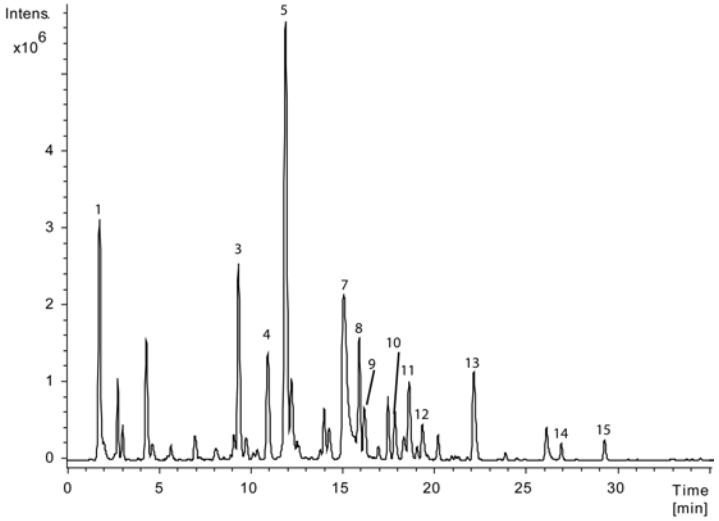
Figure S3. Overlaid HSQC and HMBC spectra of **8** (HSQC: methylene (light green), methyl and methine (dark green); HMBC: orange).



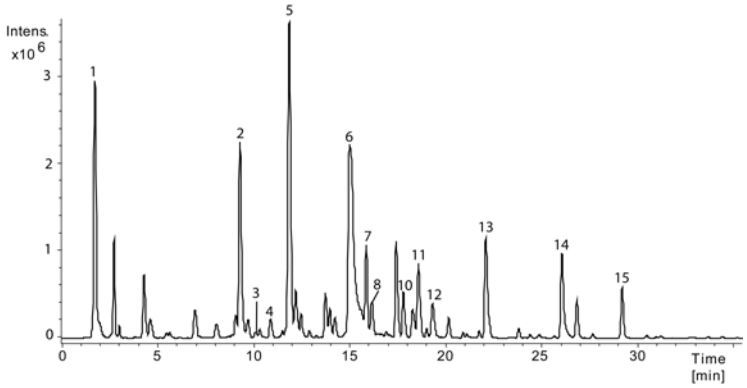
***Phyteuma orbiculare* L.**



***Phyteuma hemisphaericum* L.**



***Phyteuma spicatum* L.**



***Phyteuma ovatum* L.**

Figure S4. HPLC-ESIMS Chromatograms of the methanol extracts of flowers of different *Phyteuma* species.

Base peak chromatograms, negative ion mode.

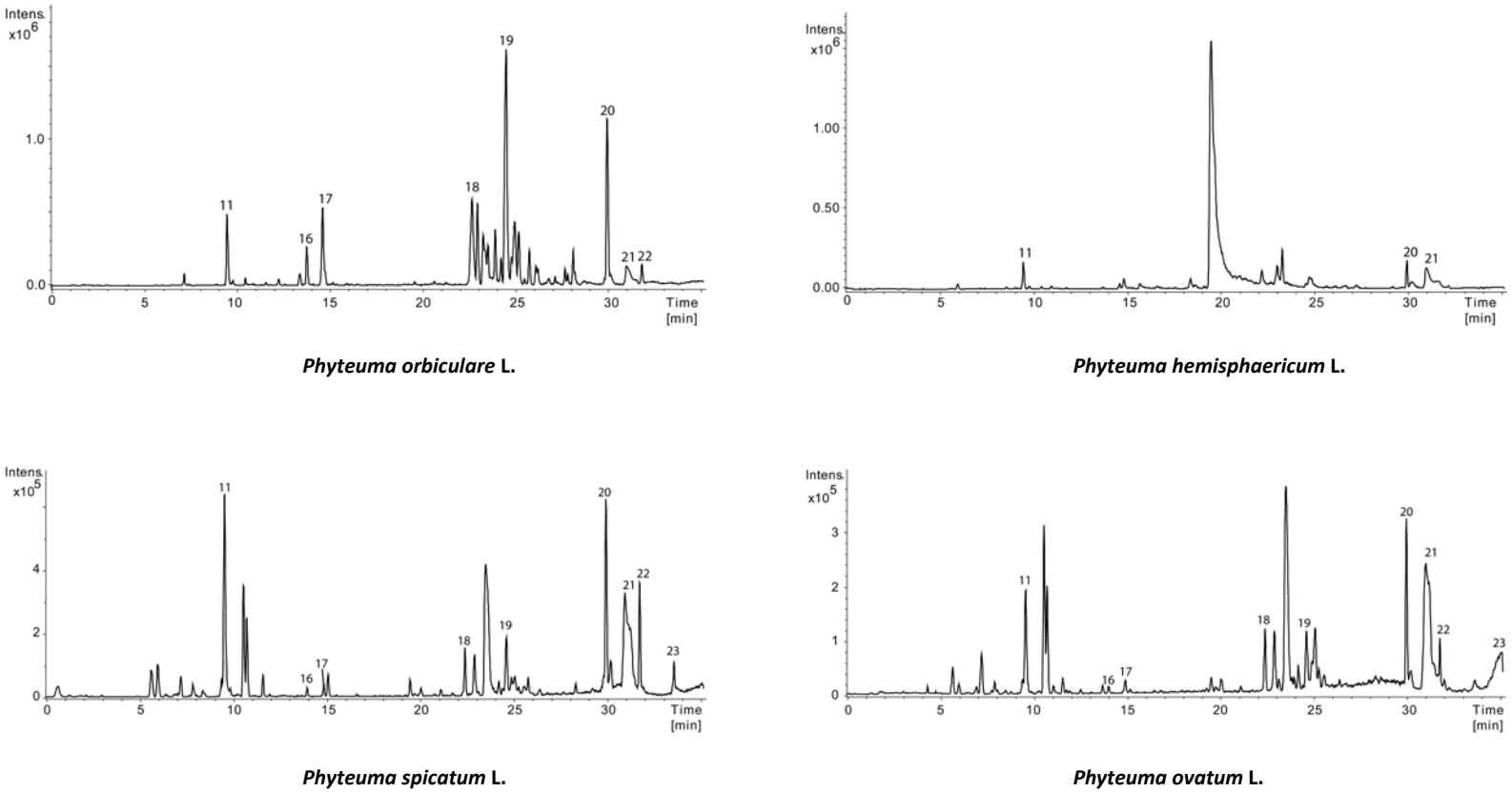
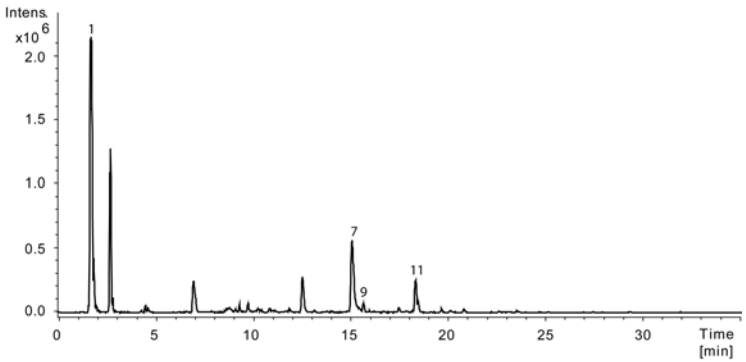
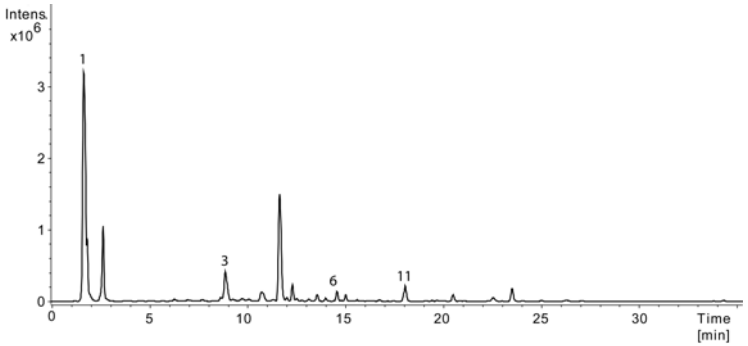


Figure S5. HPLC-ESIMS Chromatograms of the dichloromethane extract of flowers of different *Phyteuma* species.

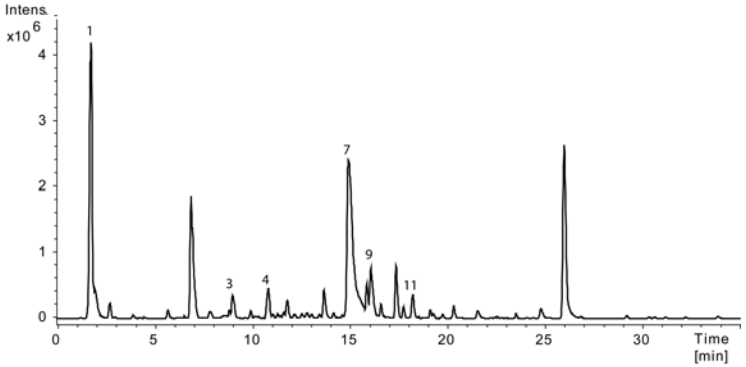
Base peak chromatograms, negative ion mode.



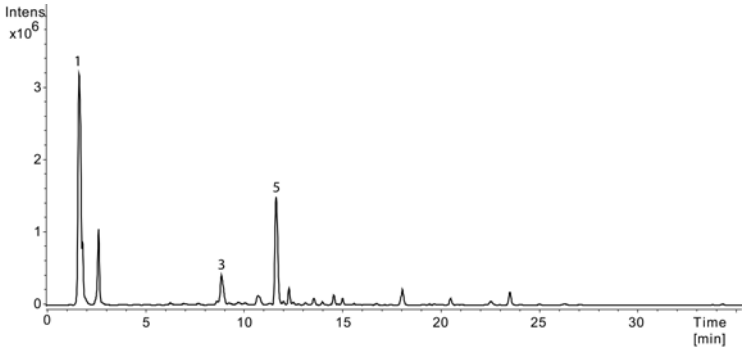
***Phyteuma orbiculare* L.**



***Phyteuma hemisphaericum* L.**



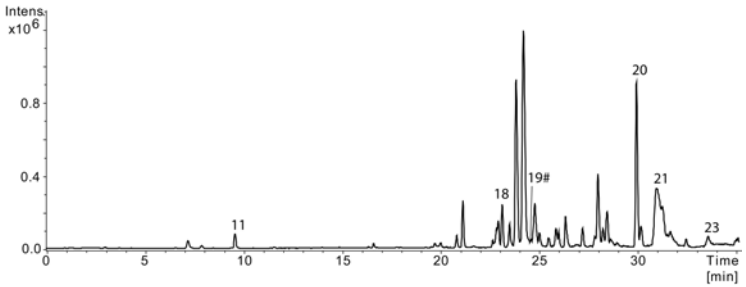
***Phyteuma spicatum* L.**



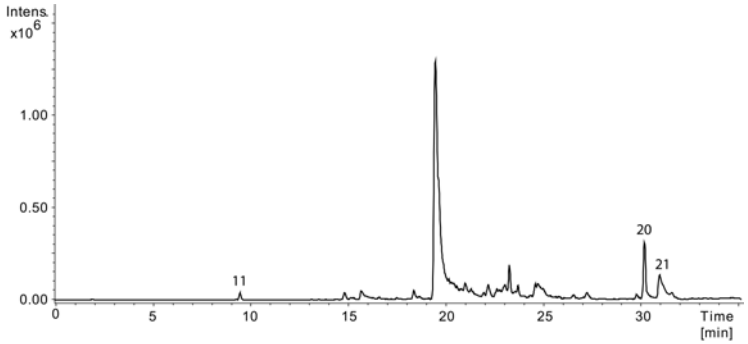
***Phyteuma ovatum* L.**

Figure S6. HPLC-ESIMS Chromatograms of the methanol extracts of leaves of different *Phyteuma* species.

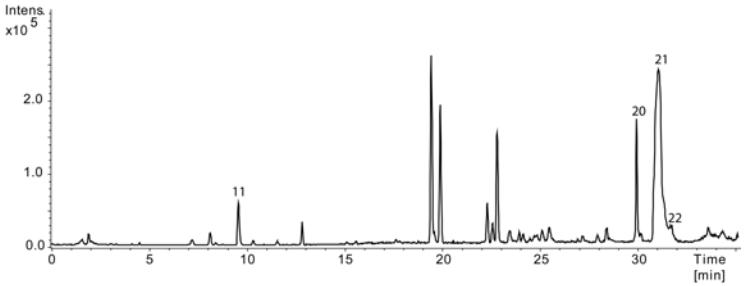
Base peak chromatograms, negative ion mode.



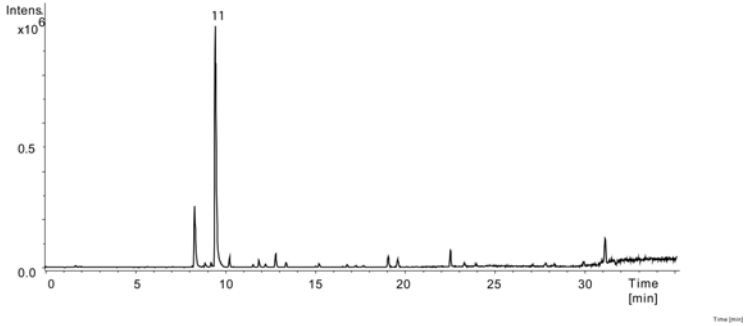
***Phyteuma orbiculare* L.**



***Phyteuma hemisphaericum* L.**



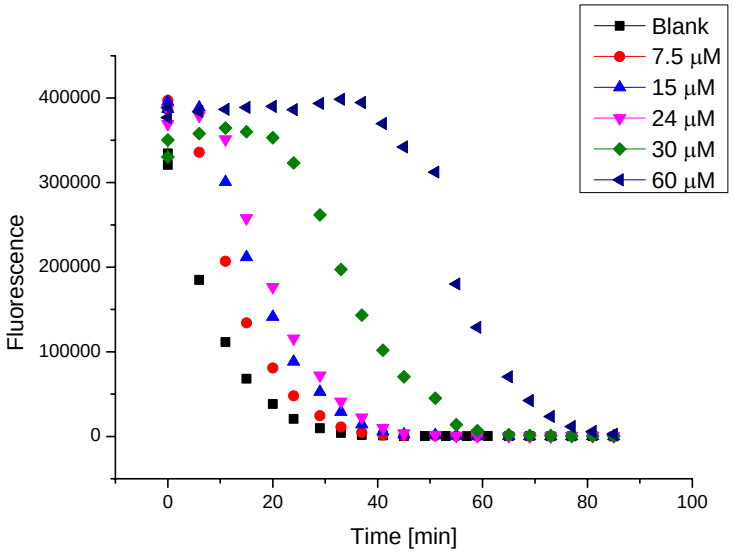
***Phyteuma spicatum* L.**



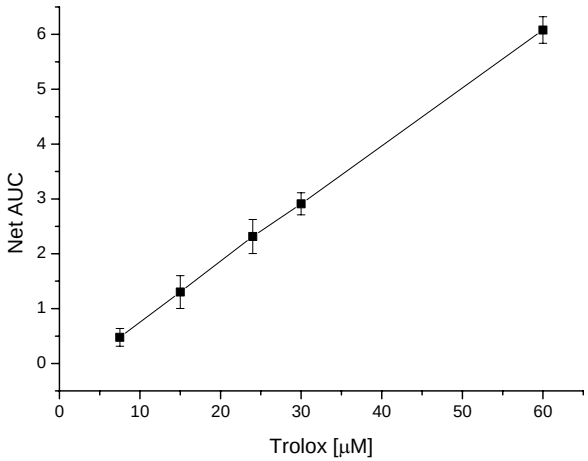
***Phyteuma ovatum* L.**

Figure S7. HPLC-ESIMS Chromatograms of the dichloromethane extracts of leaves of different *Phyteuma* species.

Base peak chromatograms, negative ion mode.



Trolox kinetic curves. Kinetic curves for Trolox® antioxidant standards ranging from 0 to 60 µM. The data were plotted using OriginLab software. The reaction was initiated by the addition of 50 µL of AAPH solution and the fluorescence monitored every four minutes using a Chameleon Multilabel Detection Platform.



Antioxidant standard curve. The net AUC of different Trolox® standards are plotted as a function of concentration. The calibration curve has been used to interpolate the antioxidant capacity of the samples of *Phyteuma orbiculare*.

Equation: $y = 0.1068x - 0.3062$ (R^2=0.9999)

Figure S8. ORAC (oxygen radical absorbance capacity) assay

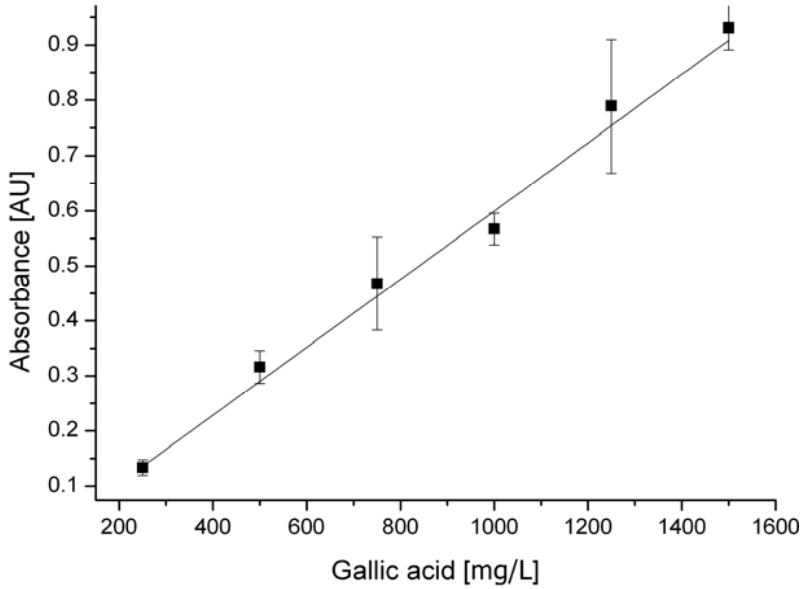


Figure S9. Calibration curve for the determination of total phenols

The Absorbance of different gallic acid standards are plotted as a function of the concentration. The resulting calibration curve has been used to interpolate the numbers of gallic acid equivalents in the samples of *Phyteuma orbiculare*.

Equation: $y = 0.0006x - 0.0192$ (R^2=0.9974)

3.3. A comprehensive metabolite profiling of *Cirsium spinosissimum* Scop.

3.3.1. Comprehensive analysis of *Cirsium spinosissimum* Scop., a wild alpine food plant

Christian Abbet, Ivan Slacanin, Elisabetta Corradi,Maria De Mieri, Matthias Hamburger, Olivier Potterat.

Food Chem. (2013); submitted.

Figure 8. *Cirsium spinosissimum* Scop.

The second plant to be investigated was the spiniest thistle (*Cirsium spinosissimum* Scop., Asteraceae, Figure 8). The genus *Cirsium* is widely used as herbal medicine or food around the world. *C. japonicum,* for example, is used in traditional Chinese medicine (TCM) as an antihemorrhagic and diuretic agent. Young stems of *C. oleracea* are eaten as vegetable in Japan and India.During the ethnobotanical investigation in canton of Valais, the spiniest thistle was reported as a plant collected to feed pigs.

In addition, this plant was traditionally eaten by shepherds similarly to an artichoke, after cutting the surrounding leaves to reach the heart of the flower, called receptacle (Figure 9).

Figure 9. Edible part of the receptacle.

Extracts of different polarities were subjected to a comprehensive metabolite profiling using a dereplication platform combining HPLC-PDA-MS and offline microprobe NMR analyses. A wide range of compounds including flavonoid glycosides, phenylpropanoids, a monoterpene lactone, fatty acids, and a spermine derivative were identified online or after targeted isolation. Quantitative data on fatty acids, minerals, polyphenols, and carotenes were obtained according to standard procedures described in the literature.Absence ofcytotoxicity of the ethanol extract was demonstrated on Caco-2 cells using a MTT metabolic activity assay.

No compounds with reported toxicity, or substance classes with known toxicological risks were detected. Based on its chemical composition combined with pleasant gustatory properties, *C. spinosissimum* can be considered as a safe wild food plant. Thistle thorns will be certainly a serious barrier to cultivation. Crosses with related species should be made to produce a less spiny hybrid.

Collection of the plant material, isolation, and interpretation of analytical data for structure elucidation (mass spectroscopy, microprobe NMR), quantification of major flavonoids, preparation of the figures, and redaction of the manuscript were my part to this publication. Dr. Slacanin provides quantitative data on vitamins, minerals, and fatty acids. Corradi performed the cytotoxicity assay on Caco-2 cell line. Dr. De Mieri checked all NMR-data.

Christian Abbet

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Comprehensive analysis of *Cirsium spinosissimum* Scop., a wild alpine food plant

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ABSTRACT

Plants which have been traditionally eaten by alpine populations may provide new opportunities of agricultural development for mountain regions. In this context we investigated the chemical composition of *Cirsium spinosissimum* (Asteraceae), a perennial thistle. Its receptacles were eaten by shepherds in Valais (Switzerland). Extracts of aerial parts were subjected to a comprehensive metabolite profiling, using a dereplication platform, combining HPLC–PDA–MS and offline microprobe NMR analysis. Twenty compounds, including various phenolic glycosides, a monoterpene lactone, a spermine derivative, and fatty acids, could be identified online, or after targeted isolation. The total phenolic content was determined, and the major flavonoids were quantitatively assessed in fresh receptacles by HPLC–PDA analysis. In addition, substances relevant for nutrition, such as β-carotene, fatty acids, ascorbic acid, and minerals, were quantified. The ethanolic extract of the receptacles showed no sign of cytotoxicity when tested in Caco-2 cells.

1. Introduction

According to modern nutritional studies, the consumption of leafy vegetables brings numerous health benefits, and their everyday consumption in diet is highly recommended (Block, 1991). Ancient food plants with pleasant gustatory properties may represent interesting opportunities for a diversified diet. Rocket salad (*Eruca sativa*) is a case in point for the successful development of a locally used food plant into a mainstream salad (Sastry, 2003). The alpine regions of Europe have a rich ancestral tradition with regard to the consumption of wild plants as medicines or food (Pieroni & Giusti, 2009; Vitalini et al., 2013). However, this traditional knowledge tends to get lost as it has been mainly transmitted orally. We recently carried out an ethnobotanical survey in Lower and Central Valais (Switzerland) to collect information on traditionally used fruits, vegetables and spices (Abbet et al., 2014). During semi-structured interviews with informants, we discovered that the receptacles of some Asteraceae, such as *Cirsium spinosissimum* Scop. were eaten like artichokes. Shepherds prepared the edible parts of the receptacle by removing the surrounding spiny leaves (Abbet, Hamburger, Potterat, & Slacanin, 2012; Mayor, 2002). Neither phytochemical nor biological data were available on *C. spinosissimum*, even though studies were reported on several other *Cirsium* species (Jordon-Thaden & Louda, 2003; Miyaichi, Matsuura, & Tomimori, 1995). We here report a comprehensive phytochemical profiling of the aerial parts of *C. spinosissimum*. In addition, we provide quantitative data on substances relevant for nutrition in receptacles, such as ascorbic acid, β-carotene, minerals, and fatty acids. Total phenolic compounds were determined, and the major phenolic constituents quantitatively assayed by HPLC–PDA analysis. Finally, a preliminary assessment of cytotoxicity was done with the Caco-2 cell line.

2. Material and methods

2.1. Plant material

Aerial parts of *C. spinosissimum* Scop. were collected by C. Abbet on 18th July 2009 in la Dotze (2300 m), near Orsières, Valais, Switzerland. The plants were identified by C. Rey, retired Senior Scientist at the Agroscope Changins-Wädenswil ACW research station in Conthey, Switzerland. A voucher specimen (nr. 551) is deposited at the Division of Pharmaceutical Biology, University of Basel, Switzerland.

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0308-8146/

2.2. Chemicals and reference compounds

Solvents were from Scharlau (Barcelona, Spain). For extraction and column chromatography (CC), technical grade solvents were used after distillation. HPLC grade solvents were employed for HPLC. HPLC grade water was obtained from an EASY-pure II (Barnstead, Dubuque IA, USA) water purification system. Deuterated solvents were purchased from Armar Chemicals (Döttingen, Switzerland). Sephadex LH-20 was purchased from GE Healthcare (Fairfield CT, USA). Diaion HP-20 resin was obtained from Sigma–Aldrich (Buchs, Switzerland). Other chemicals used were of analytical grade. Linoleic acid (**8**), α-linolenic acid (**9**), palmitic acid (**10**), chlorogenic acid (**12**), and quercetin-3-*O*-β-glucopyranoside (**16**) were purchased from Sigma–Aldrich. Malyngic acid (**5**) and pinellic acid (**6**) were previously isolated from *Phyteuma orbiculare* L. (Abbet et al., 2013). Linarin (**3**) was purchased from Extrasynthese (Lyon, France).

2.3. General experimental procedures

Pressurized liquid extraction (PLE) was carried out on an ASE 200 instrument (Dionex, Sunnyvale, CA, USA) in 22 ml steel cartridges. The following conditions were used: preheat time of 1 min, 100% cell volume flush, 80 s purge with nitrogen, pressure 120 bar.

Preparative HPLC was performed on a PuriFlash® 4100 system (Interchim, Montluçon, France) coupled to an evaporative light scattering detector (ELSD) Series 2000 (Alltech, Deerfield Il, USA, nitrogen flow 2.4 l/min, impactor on, 50 °C) via a Quick Split flow splitter (Interchim; split ratio 100:3) (System 1), or a Preparative Liquid Chromatograph, (Shimadzu, Kyoto, Japan) consisting of a SCL-10VP controller, LC-8A binary pumps, a UV–Vis SPD-M10A VP detector and Class-VP 6.12 software (System 2). Separations were performed on a SunFire Prep C_{18} OBD (30 × 150 mm, 5 µm) column (Waters, Milford, MA, USA) equipped with a precolumn (20 × 10 mm i.d.). Water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B) were used as mobile phase. The flow rate was 30 ml/min (System 1) or 20 ml/min (System 2). Semi-preparative HPLC was performed on an Agilent 1100 series instrument equipped with a photodiode array detector (PDA) (Agilent Technologies, Waldbronn, Germany). Separations were carried out at 25 °C on a SunFire C_{18} column (5 µm, 150 × 10 mm i.d., Waters) equipped with a precolumn (10 × 10 mm i.d.). Gradients of water and acetonitrile or methanol were used. The flow-rate was 3 or 4 ml/min. For the purification of **20**, an Esquire 3000 plus mass spectrometer (Bruker Daltonics, Bremen, Germany) connected via a Quick Split flow splitter (Analytical Scientific Instruments, split ratio 200:1) was used as detector. A make-up flow was delivered to the MS-line (0.5 ml/min) by a HPLC pump (Young Lin, Anyang, Korea).

NMR spectra were recorded on a 500 MHz Avance III™ spectrometer (Bruker BioSpin) equipped with a 1 mm TXI microprobe (^{1}H- and 2D-NMR) or a 5 mm BBO probe (^{13}C-NMR). Standard pulse sequences of the software package Topsin 3.0 were used. Electrospray mass spectroscopy (ESI-MS) spectra were recorded on an Esquire 3000 plus mass spectrometer (Bruker Daltonics).

2.4. Extraction and isolation

The dried aerial parts were ground using a ZM 1 ultracentrifugal mill (Retsch, Haan, Germany), with 0.75 mm Conidur sieve. The powder (256 g) was extracted at r.t. with dichloromethane (3 × 3 L, each 24 h), followed by methanol (3 × 2 L, each 24 h). The extracts were evaporated to dryness under reduced pressure to afford 14.2 g of dichloromethane extract, and 54.6 g of methanol extract.

A portion (5.9 g) of the dichloromethane extract was suspended in methanol (400 ml) and partitioned with *n*-hexane (7 × 400 ml). The methanol-soluble fraction (1.33 g) was separated on a Sephadex LH-20 column (7 × 100 cm i.d.) eluted with methanol. Eight fractions (Frs. 1-8) were collected. Fractions 4, 6, and 7 were further separated by preparative HPLC (System 1) with a gradient of 5–100% B in 30 min (see Section 2.3). Fraction 4 (170 mg) yielded **7** (4 mg), **8** (12 mg), **9** (8 mg), and **10** (7 mg); compounds **1** (1 mg), **2** (1 mg), and **7** (3 mg) were obtained from Fraction 6 (61 mg); Fraction 7 (27 mg) afforded a mixture of **3** and **4** (2 mg).

A portion (53.0 g) of the methanol extract (54.6 g) was dissolved in 300 ml of water and loaded onto a Diaion HP-20 column (70 × 400 mm i.d.) eluted with water (30 L), followed by methanol (15 L). A solid (2.7 g) precipitated upon concentration of the methanolic fraction to 1 L. 30 mg of the solid were submitted to semi-preparative HPLC to give **3** (3 mg) and **4** (2 mg). After evaporation to dryness, 5.0 g of the soluble part of the methanol fraction (8.1 g) were separated on a Sephadex LH-20 column (7 × 100 cm i.d.) eluted with methanol. 12 fractions (Frs. 1-12) were collected. A portion (100 mg) of Fraction 9 (825 mg) was submitted to preparative HPLC (System 1) to give **1** (3 mg). Fractions 11 (233 mg) and 12 (342 mg) were purified by preparative HPLC (System 2). Fraction 11 yielded compounds **12** (2 mg), **14** (10 mg), **17** (2 mg), and two mixtures which afforded compounds **18** (1 mg) and **19** (1 mg) upon final purification by semi-preparative HPLC. Likewise, Fraction 12 (342 mg) gave compound **11** (6 mg) and two mixtures which were further separated by semi-preparative HPLC to afford compounds **13** (3 mg), **15** (1 mg) and **18** (3 mg). Compound **20** (2 mg) was purified from Fr. 8 (27 mg) by semi-preparative HPLC. HPLC chromatograms are available in Supplementary Information Data (Data D1 and Data D2).

2.5. HPLC–PDA–MS analyses

HPLC–PDA–MS analyses were performed on an Agilent series 1100 system consisting of a binary pump, a column oven and PDA detector (Agilent Technologies), connected to a 215 injector (Gilson, Mettmenstetten, Switzerland) and to an Esquire 3000 plus ion trap mass spectrometer equipped with electrospray (ESI) interface (Bruker Daltonics). Separations were performed on a SunFire C_{18} column (3.5 µm, 150 × 3.0 mm i.d., Waters) equipped with a guard column (10.0 × 3.0 mm i.d., Waters). The mobile phase consisted of water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B). A gradient of 10–100% B in 30 min, then 100% B during 5 min was used for the dichloromethane extract. For the methanol extract, the following gradient was applied: 5–15% B in 5 min, 15–20% B in 10 min, 20–25% B in 20 min, 25–100% B in 17 min, then 100% B during 3 min. The flow rate was 0.4 ml/min, and the column temperature was set to 25 °C. 20 µl of a 5 mg/ml (extracts), or a 1 mg/ml (pure compounds) solution were injected. UV spectra were recorded from 200 to 400 nm. ESI-MS spectra were recorded in the negative ion mode under ion charge conditions (ICC 20000), at a scan speed of 13,000 *m*/*z*/s, using a gauss filter width of 0.2 *m*/*z*. Nitrogen was used as drying gas at a flow rate of 10 l/min, and as a nebulizing gas at a pressure of 30 psi. The nebulizer temperature was set at 300 °C. Spectra were recorded in the range of *m*/*z* 150–1500. Capillary voltage was set at −4500 v, endplate offset at 500 v, capillary exit at −128.5 v, skimmer voltage at −40 v, and trap drive at 61.4 v. Data acquisition and processing were performed using Hystar 3.0 software (Bruker Daltonics).

2.6. Quantitative analysis

2.6.1. Plant material preparation

Fresh edible parts of receptacles of *C. spinosissimum* were freeze-dried and powdered under liquid nitrogen with a M20

universal mill (Ika Werke, Staufen, Germany). The dried powder was used for all quantitative analyses. The concentrations are expressed as percent fresh weight by taking into account the moisture determined for receptacles (73.3%).

2.6.2. Fatty acid composition

2.0 g of dried powder were extracted by PLE with *n*-hexane–isopropanol (9:1) (70 °C, 3 extraction cycles of 3 min each). The extract was adjusted with *n*-hexane–isopropanol (9:1) to a final volume of 50.0 ml. The method of the European Pharmacopeia V was used for analysis (Pharmacopeia, 2005). An aliquot of extract (15.0 ml) was mixed with 2.0 ml of internal standard solution (5.0 mg/ml of methyl tricosanoate (NU-Chek Prep, Elysian, MN) in isooctane) in a quartz tube, and the solvent was evaporated under nitrogen. 1.5 ml of a 20 g/l solution of NaOH in methanol were added. The tube was filled with nitrogen and sealed with a polytetrafluoroethylene-lined cap. The reaction mixture was heated at 90 °C for 7 min. After cooling, 2 ml of BF_3–methanol (12:88) were added, and the mixture heated again at 90 °C under nitrogen for 20 min, then cooled to r.t. Isooctane (1.5 ml) was added, and the mixture was vigorously shaken. Following the addition of 5 ml of a saturated NaCl solution, the mixture was shaken and centrifuged for 5 min at 5000 rpm. The upper layer was transferred into a fresh tube, and the lower layer was extracted under shaking with 1.5 ml of isooctane. The combined isooctane extracts were washed with 1 ml of water. An aliquot of 1 µl was analyzed by GC-FID on a polyethylene glycol INNOVAX (30 m × 0.32 mm, 0.25 µm film thickness) column, with a temperature gradient of 3 °C/min from 170 to 240 °C (helium as carrier gas at a flow rate of 1.9 ml/min, split ratio (1:50)). The temperatures of injector and detector were 250 °C and 280 °C, respectively. Analyses were performed in triplicate.

2.6.3. Determination of β-carotene

1.0 g of dried powder was extracted by PLE with *n*-hexane–isopropanol (9:1) (70 °C, 3 extraction cycles of 3 min each). The extracts were analyzed by HPLC on an Agilent series 1200 system consisting of a binary pump, a column oven and a photodiode array (PDA) detector. Separations were performed on a Nucleosil 120-5 C_{18} (5 µm, 250 × 3 mm i.d.) (Macherey Nagel) column with methanol–acetonitrile–tetrahydrofuran (910:50:40) at a flow rate of 1.0 ml/min. Detection was at 452 nm. Analyses were performed in triplicate.

2.6.4. Determination of ascorbic acid

The method described in the *Manuel Suisse des Denrées Alimentaires* (MSDA) was used with slight modification (MSDA, 1992). Dried powder (about 5.0 g exactly weighed) was mixed with quartz sand and ground in a mortar with 5% metaphosphoric acid (40.0 ml) for 5 min. The mixture was filtered through a 0.25 µm PTFE filter, and the filtrate used for quantitative determination. Analyses were carried out on an Agilent series 1200 system consisting of a binary pump, a column oven and a photodiode array (PDA) detector. Separations were performed on an EC Nucleosil C18 column (5 µm, 250 × 4.0 mm i.d., Macherey–Nagel) equipped with a guard column (8.0 × 4.0 mm i.d.). The mobile phase consisted of water containing 1.03 g/l of 1-hexanesulfonic acid sodium salt (pH adjusted to 2.6 with 40% H_3PO_4) (solvent A) and acetonitrile–water (8:2) (solvent B). A gradient elution was used with 0%, 10%, 20%, 22%, 28% and 40% B at 0, 5, 12, 15, 20 and 23 min, respectively. The flow rate was 1.0 ml/min, and the column temperature was set to 25 °C. 10 µl were injected. Detection was at 241 nm. The limits of detection (LOD) and quantification (LOQ) were 0.5 mg and 2.0 mg/100 g DW, respectively. Analyses were performed in triplicate.

2.6.5. Determination of minerals

A standard operation procedure of the University of Wisconsin, Madison was used with minor modifications (University of Wisconsin-Madison, 2005). 1.0 g of dried powder was mixed with 10 ml of ultrapure concentrated HNO_3 and 1 ml of 30% H_2O_2. The mixture was shaken in a PTFE tube for 6 h at 60 °C. After centrifugation at 3000 rpm for 5 min, the supernatant was adjusted to 20.0 ml with 30% HNO_3 and finally diluted 20 times with 30% HNO_3. The minerals were quantified with an inductively coupled plasma emission spectrometer (ICPE 9000, Shimadzu, Kyoto, Japan). The following parameters were used: power 1200 W; plasma view: axial; nebulization gas: argon, 0.7 l/min; shear gas: argon, 15 l/min; auxiliary gas: argon, 0.3 l/min. The detection limit (LOD) of each element was 0.3 mg/100 g fresh weight (2 mg/100 g dried weight). Analyses were performed in triplicate.

2.6.6. Total phenolic content

200 mg of dried powder were crushed with 2 ml of 50% aqueous methanol in a nitrogen-cooled mortar. The homogenate was centrifuged (3000*g* for 15 min at 4 °C). The determination of total phenolics was done by following the standard Folin–Ciocalteau (FC) method described in the Current Protocols in Food Analytical Chemistry (Waterhouse, 2002). Briefly, 20 µl of each sample, gallic acid standards (112–1341 mg/l) or blank (water) were mixed with 1580 µl of water and 100 µl of FC solution (Sigma). The mixture was incubated for 4 min at r.t.. Then, 300 µl of a saturated sodium carbonate solution were added, and the solution was incubated for 2 h in the dark at room temperature. 200 µl of reaction mixture were transferred into a 96 well microplate, and the absorbance was measured at 750 nm on a Chameleon multilabel detection platform (Hidex, Turku, Finland). The absorbance recorded for the gallic acid standards ($n = 3$) was plotted as a function of concentration. The calibration curve ($y = 6.54\ 10^{-4} \times (r^2 = 0.9828)$) was then used to determine the number of gallic acid equivalents (GAE) in the samples ($n = 3$).

2.6.7. Quantitative determination of major flavonoids

1.0 g of dried plant material was extracted by pressurized liquid extraction (PLE) successively with dichloromethane and methanol (70 °C, 3 extraction cycles of 5 min for each solvent). HPLC analyses were performed, in triplicate, on an Alliance 2695 instrument (Waters) equipped with a 996 PDA detector. Separations were performed on a SunFire C_{18} column (3.5 µm, 150 × 3.0 mm i.d., Waters) equipped with a guard column (10.0 × 3.0 mm i.d.). The flow rate was 0.5 ml/min. The mobile phase consisted of water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B). A gradient of 20–25% B in 30 min, and to 95% B in 10 min was used. Equilibration time between the injections was 10 min. The injection volume was 10 µl. Samples were dissolved in DMSO at a concentration of 20 mg/ml for extracts, and 0.125–2.000 mg/ml for references. Linarin (**3**) was of commercial origin, while pectolinarin (**4**) was isolated from *C. spinosissimum* in the course of this study. Detection was at 254 nm. Calibration curves were used to determine the concentration of the respective compounds in the extracts. Pectolinarin: $y = 8.59\ 10^6x + 7.70\ 10^4$ ($r^2 = 0.9989$); linarin: $y = 1.60\ 10^5x + 8.68\ 10^4$ ($r^2 = 0.9998$).

2.7. Cytotoxicity assay

Fresh edible parts of receptacles were freeze-dried and milled. Aliquots of powdered material (2.5 g) were extracted by PLE with 98% EtOH at 70 °C. The solvent was removed under reduced pressure, and the extract redissolved in DMSO at a concentration of 20 mg/ml.

Caco-2 cells (passage 63) were cultured in DMEM medium supplemented with 10% fetal bovine serum, 1% non-essential amino

acids, 1% 200 mM L-glutamine, at 37 °C, and 5% CO_2. At 80% confluence, 0.25% trypsin–EDTA solution was used to harvest and passage cells. Culture medium was replaced every other day.

A colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-biphenyl tretrazolium bromide) metabolic activity assay was used to determine cell viability upon exposure to *C. spinosissimum* extracts. Briefly, Caco-2 cells were harvested, counted, and seeded in a 96-well plate for 48 h (final cell density: 5000 cells/well, total medium volume of 200 µl). After 24 h, 100 µl of medium were replaced. For the assay, 100 µl of medium were removed from each well and replaced with freshly prepared extract dilutions in DMEM medium. Final extract concentrations in the assay ranged from 3.9 to 500 µg/ml ($n = 6$). Sahandol, (Ebrahimi et al., 2013), was used as positive control at final assay concentrations of 3.12–100 µg/ml ($n = 2$). After 72 h of incubation, MTT (5 mg/ml in sterile PBS) was added to each well (20 µl), and the plate was placed on an orbital shaker at 37 °C. The supernatant was removed after 4 h, and 150 µl DMSO were added to each well to dissolve the formazan salt at 37 °C overnight. The absorbance was measured at 570 nm on a Chameleon multilabel detection platform (Hidex, Turku, Finland) and corrected for cellular background (reading at 620 nm).

3. Results and discussion

3.1. Profiling of the secondary metabolites of C. spinosissimum

To obtain a detailed picture of the metabolite profile of *C. spinosissimum*, the dried aerial parts were extracted successively with dichloromethane and methanol, and both extracts submitted to HPLC–PDA–MS analysis.

The HPLC–ESI-MS chromatogram of the dichloromethane extract revealed the presence of several non-UV-absorbing peaks (Fig. 1) which could be assigned to fatty acids and to a monoterpene lactone. 9,12,13-trihydroxy-10,15-octadecaenoic acid (**5**), 9,12,13-trihydroxy-10,15-octadecadienoic acid (**6**), α-linolenic acid (**8**), linoleic acid (**9**), and palmitic acid (**10**) were identified from ESI-MS data, and by co-chromatography with authentic samples. Four further peaks were assigned, after small-scale purification and off-line NMR analysis, to lawsonioside B (**1**) (Cuong et al., 2010), loliolide (**2**) (Kimura & Maki, 2001), and 13-hydroxy-cis-9, trans-11-octadecadienoic acid (**7**) (Tallent, Harris, Wolff, & Lundin, 1966). Their ^{1}H- and 2D NMR data were in agreement with literature values.

HPLC–PDA–ESI-MS analysis of the methanolic extract (Fig. 3) revealed a complex pattern. Two major peaks in the UV-trace (Fig. 3B

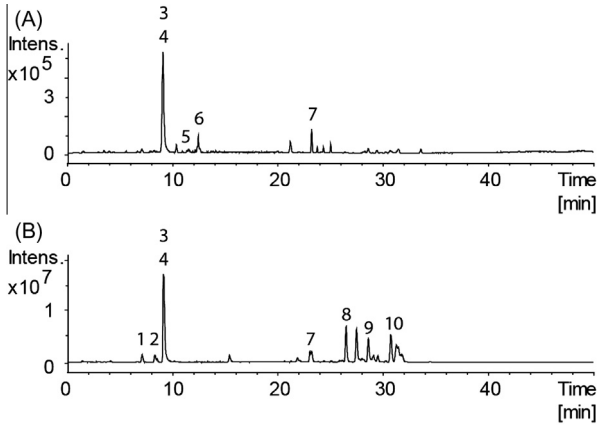

Fig. 1. HPLC–ESI-MS analysis of the dichloromethane extract. (A) base peak chromatogram, negative ion mode, m/z 150–1500; (B) base peak chromatogram, positive ion mode, m/z 150–1500.

were attributed to the flavonoids linarin (**3**) (m/z 637, $[M + HCOO]^-$) and pectolinarin (**4**) (m/z 667, $[M + HCOO]^-$) after isolation by semi-preparative HPLC. These compounds are characteristic metabolites of the genus *Cirsium* (Jordon-Thaden & Louda, 2003; Lim et al., 2008). Several additional constituents were detected, among them some that were only visible in the MS-trace (Fig. 3A). They were assigned to flavonoid glycosides, quinic acid derivatives, phenolic glucosides, a monoglyceryl glycoside, and a spermine derivative after targeted purification with the aid of gel chromatography, preparative, and semi-preparative HPLC (Fig. 2). Chlorogenic acid (**11**) (Abbet et al., 2013), 3-*O*-caffeoylquinic acid methyl ester (**12**), quercetin 3-*O*-rutinoside (**13**) (Azab, Abdel-Daim, & Eldahshan, 2013), quercetin 3-*O*-neohesperidoside (**14**) (Zhou et al., 2005), apigenin 7-*O*-rutinoside (**15**) (Fan & Yue, 2003), quercetin 3-*O*-glucopyranoside (**16**), 3-*O*-*p*-coumaroylquinic acid methyl ester (**17**) (Ohmoto & Yamaguchi, 1988), ibotanolide (**18**) (Kikuchi, Yamauchi, Nagaoka, Sugiyama, & Takahashi, 1988), N_1, N_5, N_{10}, N_{14}-tetra-*p*-coumaroylspermine (**19**) (Ma, Nakamura, & Hattori, 2001), and gingerglycolipid A (**20**) (Oliveira et al., 2012) were identified on the basis of UV, ESI-MS, ^{1}H- and ^{13}C-NMR data, and by comparison with literature values or reference compounds.

3.2. Ascorbic acid, β-carotene, fatty acids and minerals

Substances relevant for nutrition, including ascorbic acid, β-carotene, fatty acids and minerals were quantified in the fresh edible receptacles. The fatty acid composition was determined by GC-FID analysis after saponification of an *n*-hexane–isopropanol (9:1) extract. The major fatty acids were 18:3n3 (α-linolenic acid), 18:2n2 (linoleic acid), and 16:0 (palmitic acid) (Table 1). The receptacles possessed about 50% more omega 6 (12.4 ± 1.01 mg/100 g fresh weight (FW)) than omega 3 (8.93 ± 0.78 mg/100 g FW) fatty acids. The content of fatty acids in *C. spinosissimum* is low in comparison with some other wild vegetables (mean value ω3 sum: 60 mg/100 g FW; mean value ω6 sum: 29 mg/100 g FW, $n = 48$) (Cho et al., 2007). Interestingly, the content found in the receptacles of *C. spinosissimum*, and the ω6/ω3 ratio are similar to those reported for the leaves of *Cynara cornigera* (ω3 sum: 3 mg/100 g FW; ω6 sum: 8 mg/100 g FW) (USDA., 2011).

The β-carotene content in fresh receptacles (1426 ± 72 µg/100 g FW) is within the average range reported for vegetables, and close to the content in raw endive (1300 µg/100 g FW) (USDA, 2011). It is noteworthy that cooked artichoke contains only traces of β-carotene (12 µg/100 g FW) (USDA, 2011).

Ascorbic acid is relevant in collagen synthesis, and as an antioxidant (Smirnoff, 1996). The content in receptacles of *C. spinosissimum* was low (2.2 ± 0.1 mg/100 g FW) when compared to the average content reported for green leafy vegetables (33.6 mg/100 g) (Cho et al., 2007).

With regard to minerals, the receptacles of *C. spinosissimum* were found to be rich in calcium (298 ± 3.1 mg/100 g FW), potassium (506 ± 12.2 mg/100 g FW), and magnesium (40.1 ± 0.4 mg/100 g FW) (Table 1). In comparison to cooked artichoke, the fresh receptacles of *C. spinosissimum* contain approx. 15 times more calcium, 1.5 times more potassium, and the same amount of magnesium (USDA, 2011). According to the dietary reference intakes (DRI) for an adult established by the US Food and Nutrition Board of the Institute of Medicine, 100 g of receptacles would meet 30% of the daily intake for calcium, 10% for potassium, and 10% for magnesium (IOM., 2010). Heavy metals were not detected.

3.3. Total polyphenols, and quantification of the main flavonoids

The concentration of total phenolic compounds was calculated as gallic acid equivalents (GAE). Values of 410 ± 33 mg GAE/100 g FW (cv = 8%) were determined in fresh receptacles.

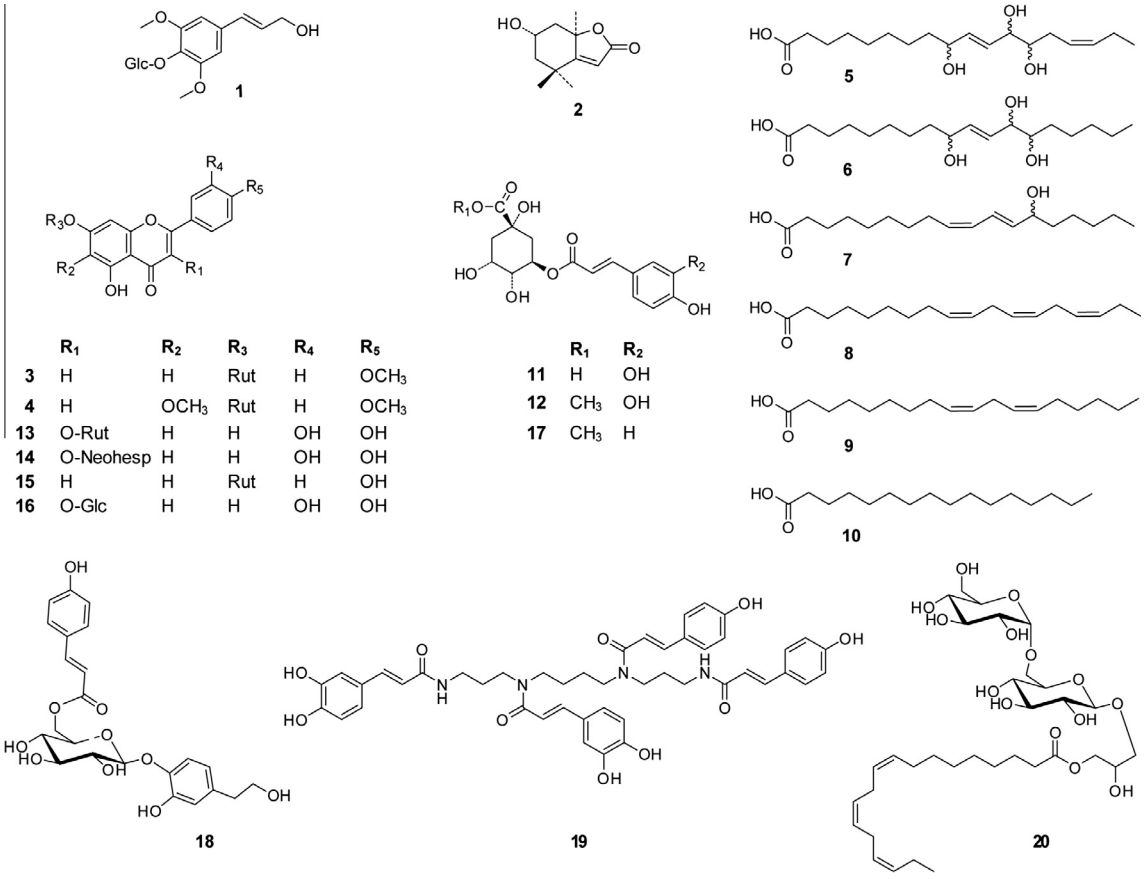

Fig. 2. Structures of the compounds identified in *C. spinosissimum*. Neohesp: neohesperoside, Rut : rutinose.

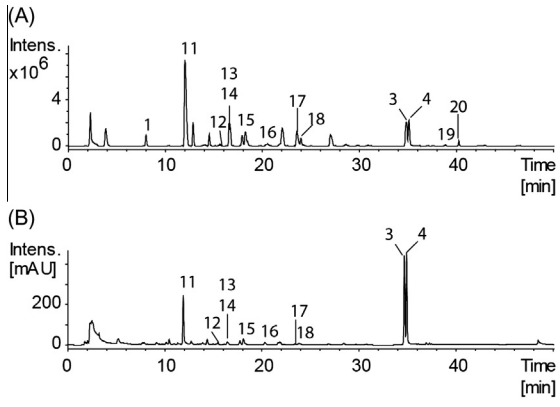

Fig. 3. HPLC–PDA–ESI-MS analysis of the methanol extract of dried aerial parts of *C. spinosissimum*. (A) ESI-MS, base peak chromatogram, negative ion mode, *m/z* 150–1500; (B) UV 254 nm. No additional peaks were detected when the UV trace was recorded at wavelengths down to 220 nm.

Table 1
Contents of substances with relevance for nutrition in receptacles of *C. spinosissimum*.

Substances	Amounts*	Substances	Amounts*
Palmitic acid	11.6 ± 0.4	Chromium	nd
Linoleic acid	12.4 ± 1.0	Copper	nd
α-Linolenic acid	8.9 ± 0.8	Iron	1.9 ± 0.2
Ascorbic acid	2.2 ± 0.1	Gallium	nd
β-Carotene	1.4 ± 0.1	Potassium	506 ± 12.2
Pectolinarin	107 ± 0.5	Lithium	nd
Linarin	85.5 ± 0.2	Magnesium	40.1 ± 0.4
Aluminium	nd	Sodium	2.7 ± 0.2
Boron	1.0 ± 0.1	Nickel	nd
Barium	nd	Phosphor	71.7 ± 1.5
Beryllium	nd	Manganese	0.7 ± 0.1
Bismuth	nd	Lead	nd
Calcium	298 ± 3.1	Selenium	nd
Cadmium	nd	Strontium	0.8 ± 0.1
Cobalt	nd	Zinc	0.8 ± 0.1

* Amounts are expressed in mg/100 g fresh weight. nd: not detected.

Compared to the leaves of four other species from the genus *Cirsium* the polyphenol content in the dried receptacles of *C. spinosissimum* was two to four times lower (Nazaruk et al., 2008).

Linarin (**3**) and pectolinarin (**4**) are typical flavonoids of the genus *Cirsium* (Miyaichi et al., 1995). Their content in fresh receptacles was determined by HPLC–PDA analysis (Table 1) after optimization of the HPLC conditions (see Supplementary information Data D3). The amount of pectolinarin (**7**) was 107 ± 0.5 mg/100 g FW (5.73 ± 0.03 mg/g DW), and that of linarin 85.5 ± 0.2 mg/100 g FW (4.56 ± 0.01 mg/g DW). These values are in agreement with data already reported for other *Cirsium* species. For example, the amount of linarin varies from 1.52 mg/g DW to 21.2 mg/g DW in *C. setidens* and from 2.6 to 11.5 mg/g DW in *C. japonicum*, while contents of pectolinarin are between 3.1 and 20 mg/g DW in *C. japonicum* (Ganzera, Pocher, & Stuppner, 2005; Lu et al., 2009; Sun et al., 2012). Large amounts of linarin and pectolinarin may have some medicinal relevance, since these flavonoids showed significant anti-inflammatory activity in various *in vivo* models, and anti-allergic and analgesic properties (Lim et al., 2008; Martinez-Vazquez, Apan, Lastra, & Bye, 1998).

3.4. Cytotoxicity

To qualify as a food plant, *C. spinosissimum* must be devoid of toxicity. Preliminary cytotoxicity assessment was performed with Caco-2 cells. No cytotoxicity was detected at concentrations up to 500 μg/ml of ethanolic extract. Similar studies, conducted with other *Cirsium* species, also showed absence of cytotoxicity at high extract concentration (Borawska et al., 2010; Ozcelik et al., 2005).

4. Conclusions

This study represents the first phytochemical analysis of *C. spinosissimum*, a wild edible plant of the Valais region. Various types of secondary metabolites were identified. No compound with reported toxicity, nor substance classes with known toxicological risks, were detected. No cytotoxicity was observed in an *in vitro* assay on Caco-2 cells.

At the same time, *C. spinosissimum* may possess interesting nutritive properties. The large amounts of potassium, calcium, and magnesium could help to meet the recommended dietary intakes of these minerals. Several pharmacological studies have shown positive effects of the two major flavonoids linarin and pectolinarin. Considering the chemical composition and pleasant gustatory properties of the receptacles, *C. spinosissimum* can be considered as a safe and nutritionally valuable wild food plant which may be of interest for mountain agriculture. The spiny leaves of the plant, however, represent a challenge for cultivation and harvesting.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.03.068.

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Supporting Information

Comprehensive analysis of *Cirsium spinosissimum* Scop., a wild alpine food plant

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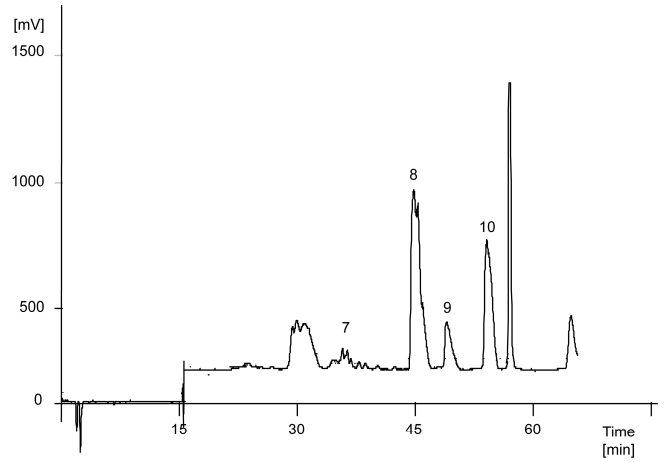
Data D1.

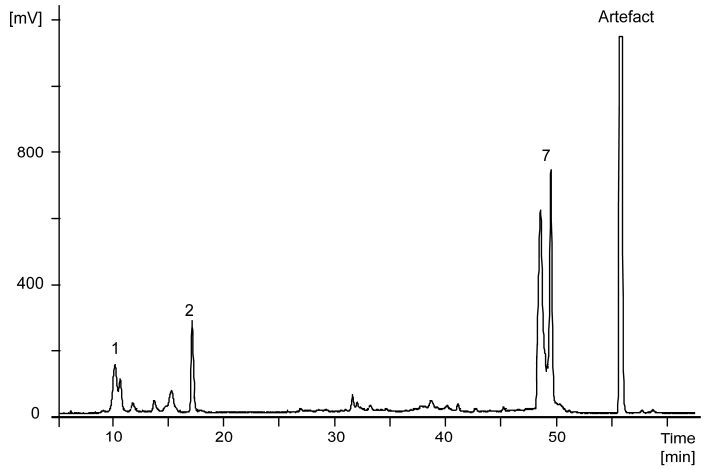
Preparative HPLC chromatograms for the separation of fractions from the dichloromethane extract.

Preparative HPLC performed on a PuriFlash® 4100 system (Interchim) coupled to an evaporative light scattering detector (ELSD) Series 2000 (Alltech; nitrogen flow 2.4 L/min, impactor on, 50 °C) via a Quick Split flow splitter (Interchim; split ratio 100:3). Separations were performed on a SunFire Prep C_{18} OBD (30 × 150 mm, 5 µm) column (Waters) equipped with a precolumn (20 x 10 mm i.d.). Water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B) were used as mobile phase. The flow rate was 30 mL/min and the gradient of 5 to 100% B in 50 min, then 100% B.

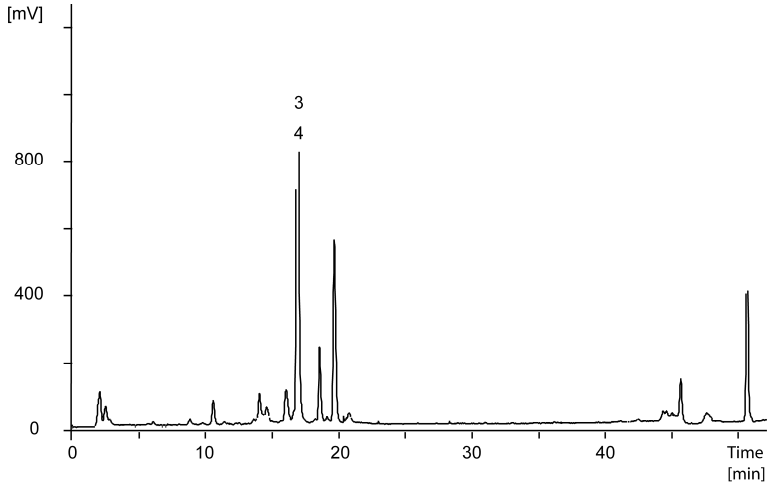
1) FRACTION 4



2) FRACTION 6



3) FRACTION 7

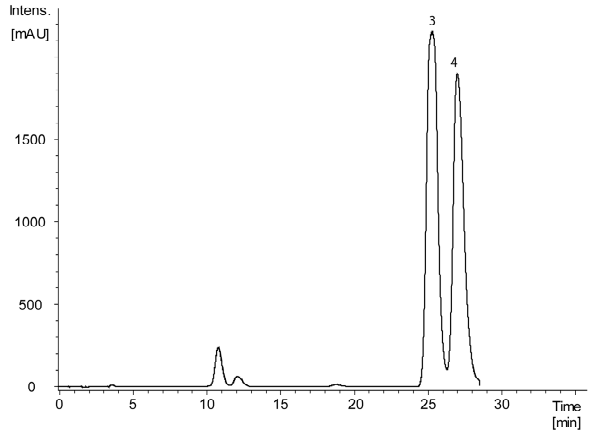


Data D2.

Preparative and semi-preparative HPLC chromatograms for the separation of fractions from the methanol extract.

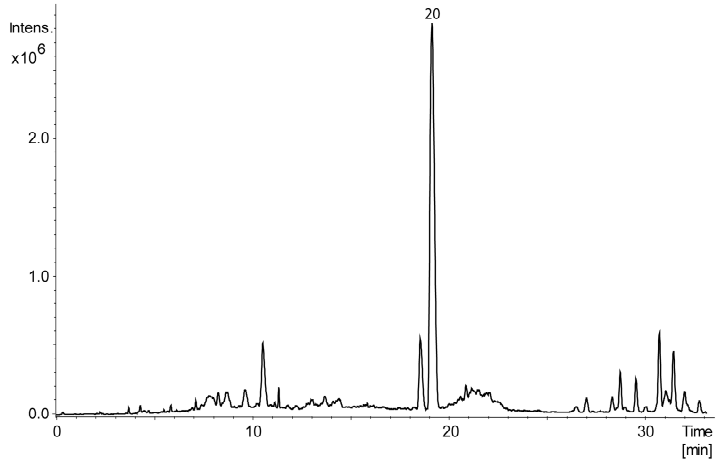
1) INSOLUBLE FRACTION

SunFire C_{18} column 5 μm, 150 x 10.0 mm I.D. (Waters) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to a DAD detector (λ=254 nm). Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeOH, flow rate: 3 mL/min, column temperature: 25 °C. Gradient: 5% to 25% B in 25 min, then to 40% B in 25 min.



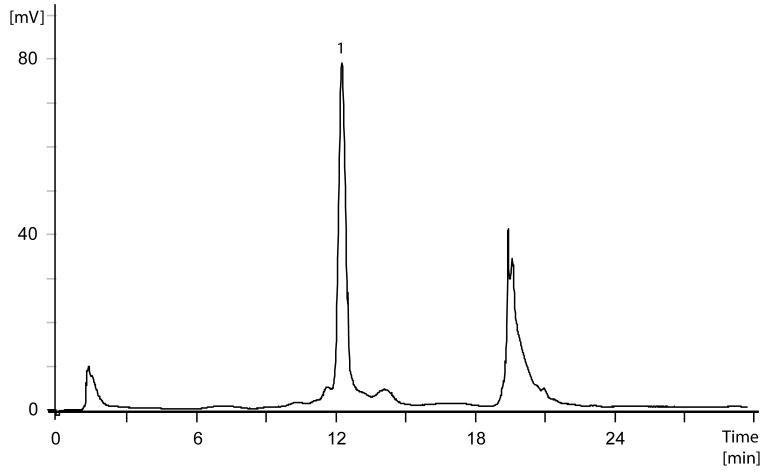
2) FRACTION 8

SunFire C_{18} column 5 μm, 150 x 10.0 mm I.D. (Waters) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to an Esquire 3000 plus mass spectrometer (Bruker) connected via a Quick Split flow splitter (Analytical Scientific Instruments, split ratio 200:1). A make-up flow was delivered to the MS-line (0.5 mL/min) by a HPLC pump (Young Lin). Spectra were recorded in the range of *m/z* 150 to 1500. Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeCN, flow rate: 4 mL/min, column temperature: 25 °C. Gradient: 10% to 100% B in 30 min.



3) FRACTION 9

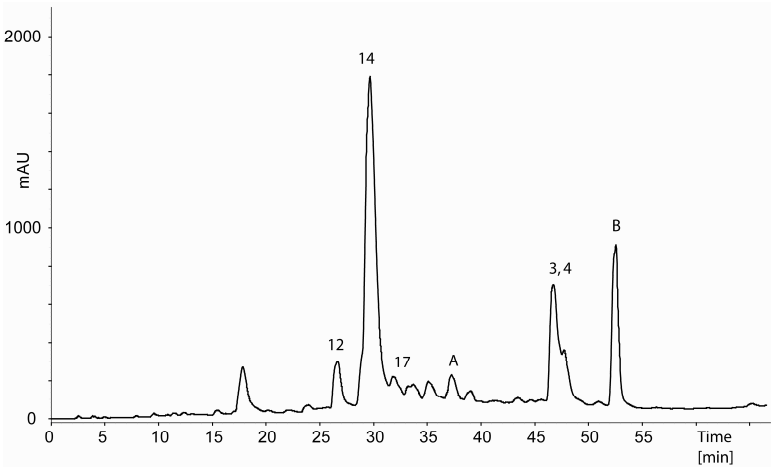
Preparative HPLC was performed on a PuriFlash® 4100 system (Interchim, France) coupled to an evaporative light scattering detector (ELSD) Series 2000 (Alltech, nitrogen flow 2.4 L/min, impactor on, 50 °C) via a Quick Split flow splitter (Interchim, split ratio 100:3) (System 1). Separations were performed on a Puriflash C_{18} HP (35 g, 15 µm) column (Interchim). Water (solvent A) and acetonitrile (solvent B) were used as mobile phase. The flow rate was 20 mL/min. Gradient was 5% of B during 5 min; then 5% to 75% of B in 15 min.



4) FRACTION 11

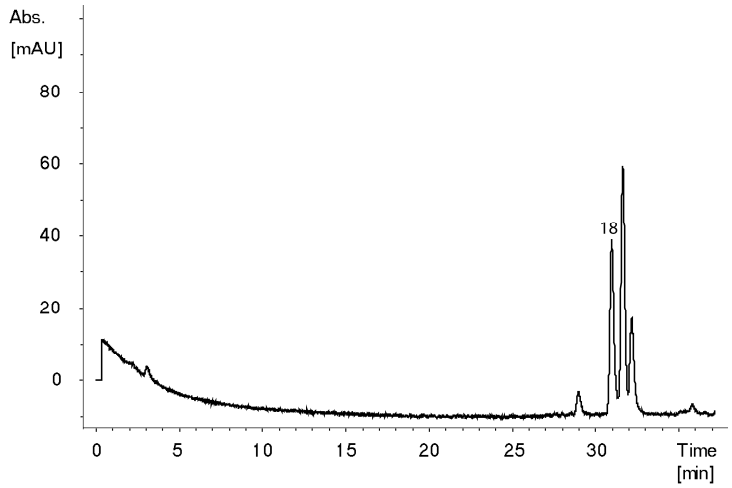
4.1) Separation of Fraction 11

Preparative HPLC was performed on a Preparative Liquid Chromatograph, (Shimadzu, Kyoto, Japan) consisting of a SCL-10VP controller, LC-8A binary pumps, a UV-Vis SPD-M10A VP detector and Class-VP 6.12 software (λ=254 nm). Separations were performed on a SunFire Prep C_{18} OBD (30 × 150 mm, 5 µm) column (Waters) equipped with a precolumn (20 x 10 mm i.d.). Water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B) were used as mobile phase. The flow rate was 20 mL/min. Gradient was 15% to 75% of B in 70 min.



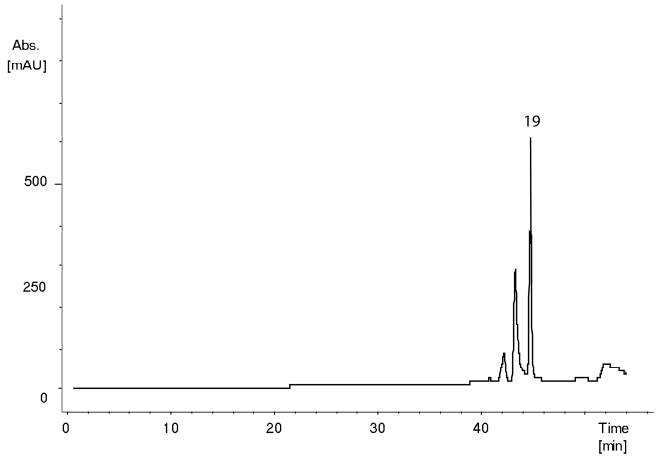
4.2) Separation of Peak A of Fraction 11

SunFire C_{18} column 5 µm, 150 x 10.0 mm I.D. (Waters) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to a DAD detector (λ=254 nm). Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeCN, flow rate: 3 mL/min, column temperature: 25 °C. The gradient was 10% to 30% of B in 40 min.



4.3) Separation of Peak B of Fraction 11

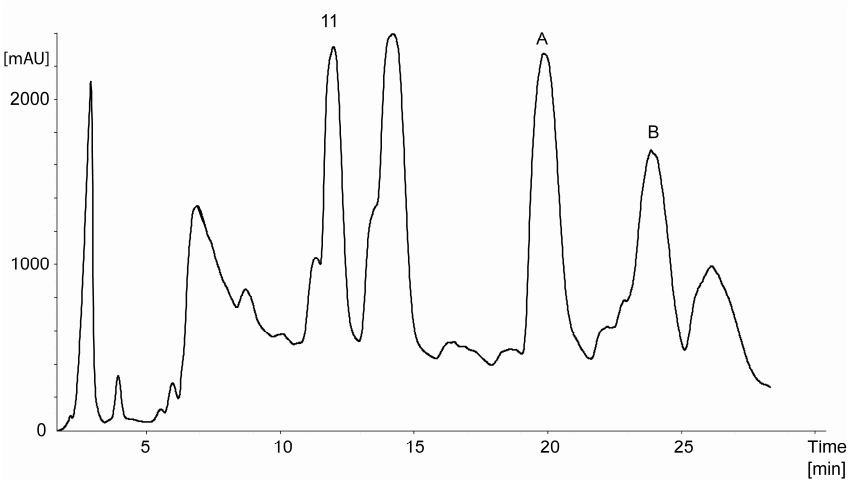
SunFire C_{18} column 5 µm, 150 x 10.0 mm I.D. (Waters) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to a DAD detector (λ=254 nm). Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeCN, flow rate: 3 mL/min, column temperature: 25 °C. The gradient was 20% to 60% of B in 50 min.



5) FRACTION 12

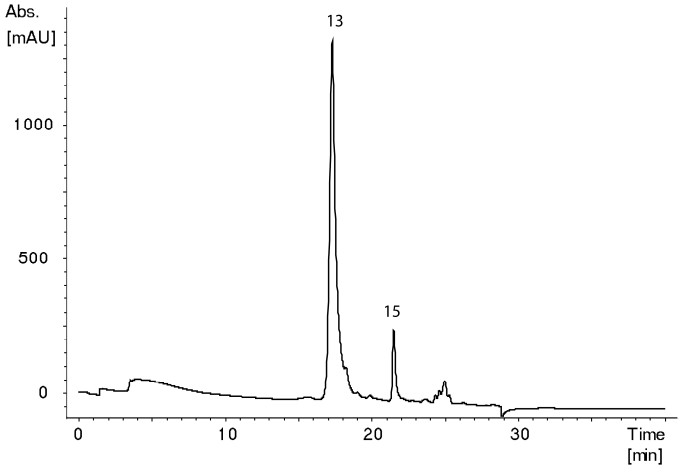
5.1) Separation of Fraction 12

Preparative HPLC was performed on a Preparative Liquid Chromatograph, (Shimadzu) consisting of a SCL-10VP controller, LC-8A binary pumps, a UV-Vis SPD-M10A VP detector and Class-VP 6.12 software (λ=254 nm). Separations were performed on a SunFire Prep C_{18} OBD (30 × 150 mm, 5 µm) column (Waters) equipped with a precolumn (20 x 10 mm i.d.). Water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B) were used as mobile phase. The flow rate was 20 mL/min. Gradient was 15% to 75% of B in 70 min.



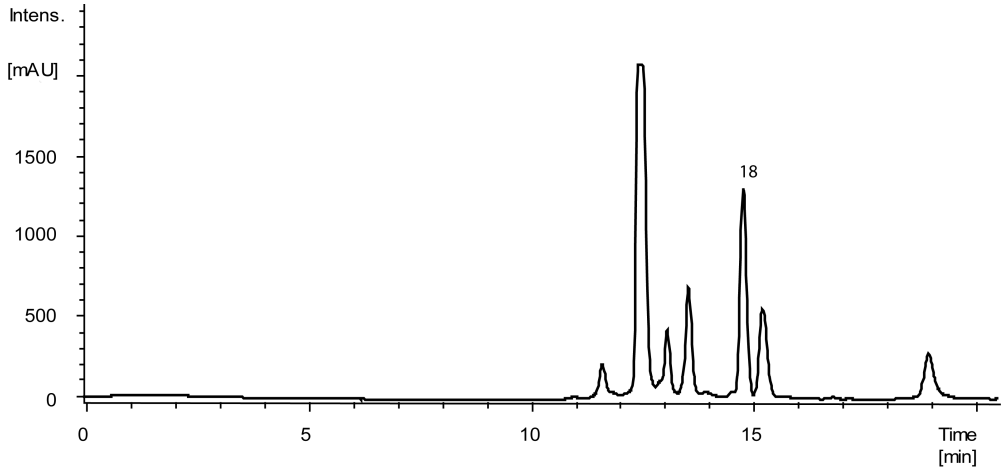
5.2) Separation of Peak A of Fraction 12

SunFire C_{18} column 5 µm, 150 x 10.0 mm I.D. (Waters) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to a DAD detector (λ=254 nm). Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeCN, flow rate: 3 mL/min, column temperature: 25 °C. The gradient was 30% to 62% of B in 40 min.



5.3) Separation of Peak B of Fraction 12

SunFire C_{18} column 5 µm, 150 x 10.0 mm I.D. (Waters) equipped with a guard column (10.0 x 10.0 mm I.D.) connected to a DAD detector (λ=254 nm). Separations conditions were as follows: mobile phase A: H_2O, mobile phase B: MeCN, flow rate: 3 mL/min, column temperature: 25 °C. The gradient was 30% to 46% of B in 20 min.



Data D3.

Physical and spectroscopic data of the isolated compounds

Lawsoniaside B (**1**). White solid. ESI-MS: *m/z* 417 $[M+HCOO]^-$. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.03 (1H, *m*, H_{Glc}-5), 3.14 (1H, *m*, H_{Glc}-4), 3.20 (2H, H_{Glc}-2, H_{Glc}-3), 3.45 (1H, *dd*, *J* =11.5, *J* = 5.3, H_{Glcb}-6), 3.61 (1H, *dd*, *J* =11.7, *J* = 1.7, H_{Glca}-6), 3.79 (6H, *s*, -OMe), 4.12 (1H, *d*, *J* = 5.0, H-9), 4.86 (1H, *d*, *J* = 6.0, H_{Glc}-1), 6.33 (1H, *dt*, *J* =16.0, J = 5.0, H-8), 6.48 (1H, *d*, *J* = 16.0, H-7), 6.68 (2H, *s*, H-2, H-6). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 56.3 (-OMe), 60.8 (C_{Glc}-6), 61.2 (C-9), 69.8 (C_{Glc}-4), 73.9 (C_{Glc}-2), 76.4 (C_{Glc}-3), 76.9 (C_{Glc}-5), 102.4 (C_{Glc}-1), 104.4 (C-2, C-6), 128.3 (C-7), 129.9 (C-8), 132.6 (C-1), 134.4 (C-4), 152.5 (C-3, C-5).

Loliolide (**2**). White solid. ESI-MS: *m/z* 219 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.25 (3H, *s*, C-9), 1.45 (3H, *s*, C-10), 1.51 (1H, *dd*, *J* =14.6, *J* = 3.7, H-2a), 1.76 (3H, *s*, C-11), 1.77 (1H, *dd*, *J* =14.5, *J* = 4.0, H-4a), 1.96 (1H, *dt*, *J* = 14.5, *J* = 2.5, H-2b), 2.43 (1H, *dt*, *J* = 14.0, *J* = 2.5, H-4b), 4.30 (1H, *q*, *J* = 3.6, H-3), 5.70 (1H, *s*, H-7). ^{13}C-NMR ($CDCl_3$, extracted from 2D-HMBC and HSQC spectra): δ 26.6 (C-10) and 27.1 (C-11), 30.7 (C-9), 36.0 (C-1), 45.8 (C-4), 47.6 (C-2), 66.8 (C-3), 87.0 (C-5), 112.9 (C-7), 172.1 (C-8), 182.7 (C-6).

Linarin (**3**). Yellow powder. ESI-MS: *m/z* 638 $[M+HCOO]^-$. UV (MeOH) λ_{max} nm: 268, 325; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.08 (3H, *d*, *J* = 6.3, H_{Rha}-6), 3.18 (2H, *m*, H_{Glc}-4, H_{Rha}-4), 3.26-3.34 (2H, *m*, H_{Glc}-2, H_{Glc}-3), 3.46 (3H, *m*, H_{Rha}-3, H_{Rha}-5, H_{Glcb}-6), 3.59 (1H, *dd*, *J* = 8.1, *J* = 7.7, H_{Glc}-5), 3.67 (1H, *dd*, *J* =3.3, *J* =1.3, H_{Rha}-2), 3.86 (3H, *s*, OMe-4'), 3.86 (1H, *d*, *J* = 11.1, H_{Glca}-6), 4.56 (1H, br *s*, H_{Rha}-1), 5.05 (1H, *d*, *J* = 7.0, H_{Glc}-1), 6.45 (1H, br *s*, H-6), 6.79 (1H, *s*, H-8), 6.85 (1H, *s*, H-3), 7.15 (2H, *d*, *J* = 8.2, H-3', H-5'), 8.05 (2H, *d*, *J* = 8.2, H-2', H-6'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 18.2 (C_{Rha}-6), 55.6 (-OCH_3-4'), 66.5 (C_{Glc}-6), 68.7 (C_{Rha}-5), 69.2 (C_{Glc}-4), 69.9-70.4 (C_{Rha}-2, C_{Rha}-3), 71.7 (C_{Rha}-4), 72.7 (C_{Glc}-2), 75.3 (C_{Glc}-5), 75.8 (C_{Glc}-3), 94.1 (C-8), 99.2 (C-6), 100.4 (C_{Glc}-1),

100.9 (C_{Rha}-1), 103.3 (C-3), 114.2 (C-3' and C-5'), 123.5 (C-1'), 127.8 (C-2' and C-6'), 162.4 (C-4'). 163.8 (C-2).

Pectolinarin (**4**). Yellow powder. ESI-MS: *m/z* 668 $[M+HCOO]^-$. UV (MeOH) λ_{max} nm: 268, 345; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.08 (3H, *d*, *J* = 6.3, H_{Rha}-6), 3.14-3.24 (2H, *m*, H_{Glc}-4, H_{Rha}-4), 3.31-3.36 (2H, *m*, H_{Glc}-2, H_{Glc}-3), 3.39-3.53 (3H, *m*, H_{Rha}-3, H_{Rha}-5, H_{Glcb}-6), 3.62 (1H, *dd*, *J* =8.1, *J* = 7.7, H_{Glc}-5), 3.67 (1H, *dd*, *J* = 1.3, *J* = 3.3, H_{Rha}-2), 3.80 (3H, *s*, OMe-6), 3.88 (3H, *s*, OMe-4'), 3.89 (1H, *d*, *J* = 11.1, H_{Glca}-6), 4.56 (1H, br *s*, H_{Rha}-1), 5.11 (1H, *d*, *J* = 6.0, H_{Glc}-1), 6.76-7.10 (2H, br *s*, H-3, H-8), 7.15 (2H, *d*, *J* = 9.0, H-3', H-5'), 8.05 (2H, *d*, *J* = 8.9, H-2', H-6'), 12.9 (1H, s, 5-OH). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 18.2 (C_{Rha}-6), 56.0 (OMe-4'), 60.1 (OMe-6), 66.5 (C_{Glc}-6), 68.7 (C_{Rha}-5), 70.0 (C_{Glc}-4), 70.7 (C_{Rha}-2), 71.3 (C_{Rha}-3), 72.5 (C_{Rha}-4), 73.5 (C_{Glc}-2), 76.1 (C_{Glc}-5), 76.8 (C_{Glc}-3), 94.8 (C-8), 100.7 (C_{Glc}-1), 100.9 (C_{Rha}-1), 103.7 (C-3), 115.1 (C-3', C-5'), 122.0 (C-1'), 128.6 (C-2',C-6'), 133.0 (C-6), 162.0 (C-4'), 164.0 (C-2).

13-Hydroxy-cis-9,trans-11-octadecadienoic acid (**7**). White solid. ESI-MS: *m/z* 295 $[M-H]^-$. UV (MeOH) λ_{max} nm: 234; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.82 (3H, *dt*, *J* = 7.0, *J* = 2.6, H-18), 1.19-1.44 (16H, *m*, H-4 to H-7, H-14 to H-17), 1.49 (2H, m, H-3), 2.09-2.21 (4H, m, H-2, H-8), 3.94 (1H, *q*, *J* = 6.0, H-13), 5.36 (1H, *dt*, *J* = 10.5, *J* =7.5, H-9), 5.65 (1H, *dd*, *J* =15.2, *J* = 6.0, H-12), 5.95 (1H, *t* , *J* = 11, H-10), 6.41 (1H, *dd*, *J* = 15.2, *J* = 11.2, H-11). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra) : δ 14.1 (C-18), 22.0 (C-17), 24.6 (C-3), 24.5-24.6 (C-5, C-15), 27.0 (C-8), 28.6-28.6-28.9 (C-4, C-6, C-7), 31.0 (C-16), 34.0 (C-2), 37.2 (C-14), 70.7 (C-13), 124.0 (C-11), 128.5 (C-10), 131.1 (C-9), 138.3 (C-12), 175.1 (C-1).

Chlorogenic acid (**11**). White powder. ESI-MS: *m/z* 353 $[M-H]^-$. UV (MeOH) λ_{max} nm: 218, 244, 324. ^{1}H-NMR (CD_3OD, 500 MHz): Caffeoyl moiety: δ 6.18 (1H, *d*, *J* = 15.0, H-8), 6.77 (1H, *d*, *J* = 7.5, H-5), 6.94 (1H, *dd*, *J* =8.1, *J* = 1.3, H-6), 7.02 (1H, *d*, *J* = 1.3, H-2), 7.41 (1H, *d*, *J* = 15.9, H-7); quinic acid moiety: δ 1.81 (1H, *dd*, *J* = 13.0, *J* =7.0, H-6a) 1.91-2.10 (3H, *m*, CH_2-2, H-6b), 3.59 (1H, *dd*, *J* = 7.0, *J* =3.0, H-

4), 3.97 (1H, *ddd*, *J* = 7.0, *J* = 3.5, *J* =3.3, H-5), 5.11 (1H, *ddd*, *J* = 6.8, *J* =5.3, *J* = 7.0, H-3). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra), caffeoyl moiety: 114.6 (C-2), 114.8 (C-8), 116.2 (C-5), 121.9 (C-6), 126.2 (C-1), 145.5 (C-7), 146.1 (C-3), 148.3 (C-4), 167.5 (C-9); quinic acid moiety : δ 37.1 (C-6), 37.7 (C-2), 70.2 (C-5), 70.7 (C-3), 72.0 (C-4).

3-O-caffeoylquinic acid methyl ester (**12**). White powder. ESI-MS: *m/z* 367 $[M-H]^-$. UV (MeOH) λ_{max} nm: 219, 244, 296 (sh), 328. ^{1}H-NMR (DMSO-d_6, 500 MHz): Caffeoyl moiety: δ 6.18 (1H, *d*, *J* = 15.0, H-8), 6.77 (1H, *d*, *J* = 7.5, H-5), 6.94 (1H, *dd*, *J* =8.1, *J* =1.3, H-6), 7.02 (1H, *d*, *J* = 1.3, H-2), 7.41 (1H, *d*, *J* = 15.9, H-7); quinic acid moiety: δ 1.81 (1H, *dd*, *J* = 7.0, *J* = 13.0, H-6a) 1.91-2.10 (3H, *m*, H-2, H-6b), 3.50-3-68 (4H, m, H-4, -CO_2Me), 3.97 (1H, *ddd*, *J* = 3.3, *J* = 3.5, *J* = 7.0, H-5), 5.11 (1H, *ddd*, *J* = 5.3, *J* = 6.8, *J* = 7.0, H-3). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra), caffeoyl moiety: 114.6 (C-2), 114.8 (C-8), 116.2 (C-5), 121.9 (C-6), 126.2 (C-1), 145.5 (C-7), 146.1 (C-3), 148.3 (C-4), 167.5 (C-9); quinic acid moiety δ 35.2 (C-2), 37.3 (C-6), 51.5 (-CO_2Me), 67.1 (C-5), 69.5 (C-4), 70.8 (C-3).

Quercetin 3-*O*-rutinoside (**13**). Yellow powder. ESI-MS: *m/z* 609 $[M-H]^-$. UV (MeOH) λ_{max} nm: 256, 355; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.08 (3H, *d*, *J* = 6.3, H_{Rha}-6), 3.00-3-18 (2H, *m*, H_{Glc}-4, H_{Rha}-4), 3.20-3-37 (6H, *m*, H_{Glc}-2, H_{Glc}-3, H_{Glc}-5, H_{Glcb}-6, H_{Rha}-3, H_{Rha}-5), 3.38 (1H, br *s*, H_{Rha}-2), 3.66 (1H, *d*, *J* = 10.5, H_{Glca}-6), 4.41 (1H, br *s*, H_{Rha}-1), 5.30 (1H, *d*, *J* = 6.0, H_{Glc}-1), 6.14 (1H, br *s*, H-6), 6.27 (1H, *s*, H-8), 6.86 (1H, *d*, *J* = 8.0, H-5'), 7.50 (1H, *d*, *J* = 1.7, H-2'), 7.51 (1H, *d*, *J* = 8.2, H-6'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 18.2 (C_{Rha}-6), 66.7 (C_{Glc}-6), 68.7 (C_{Rha}-5), 69.9 (C_{Glc}-4), 70.0 (C_{Rha}-2), 70.3 (C_{Rha}-3), 71.4 (C_{Rha}-4), 73.8 (C_{Glc}-2), 75.6 (C_{Glc}-5), 76.3 (C_{Glc}-3), 95.4 (C-8), 99.8 (C-6), 101.3 (C_{Rha}-1), 102.0 (C_{Glu}-1), 116.0 (C-5'), 116.8 (C-2'), 122.1 (C-6').

Quercetin 3-*O*-neohesperidoside (**14**). Yellow powder. ESI-MS: *m/z* 609 $[M-H]^-$. UV (MeOH) λ_{max} nm: 256, 266, 354; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.82 (3H, *d*, *J* = 6.0, H_{Rha}-6), 3.03-3.16 (3H, *m*, H_{Glc}-5, H_{Rha}-3, H_{Rha}-4), 3.27 (1H, *dd*, *J* =11.5, *J* = 5.2, H_{Glcb}-6), 3.40-3.60 (4H, *m*, H_{Glc}-2, H_{Glc}-4, H_{Glca}-6, H_{Rha}-2),

3.66-3.76 (2H, *m*, H_{Glc}-3, H_{Rha}-5), 5.09 (1H, br *s*, H_{Rha}-1), 5.62 (1H, *d*, *J* = 7.2, H_{Glc}-1), 6.16 (1H, br *s*, H-6), 6.36 (1H, br *s*, H-8), 6.81 (1H, *d*, *J* = 8.5, H-5'), 7.55 (1H, br *s*, H-2'), 7.57 (1H, *d*, *J* = 8.3, H-6'), 12.63 (1H, *br s*, OH-5). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 17.2 (C_{Rha}-6), 60.9 (C_{Glc}-6), 68.1 (C_{Rha}-5), 70.1 (C_{Rha}-3), 70.6 (C_{Rha}-2), 70.7 (C_{Glc}-3), 71.8 (C_{Rha}-4), 77.3 (C_{Glc}-5), 77.4 (C_{Glu}-4), 77.7 (C_{Glu}-2), 93.5 (C-8), 98.4 (C_{Glc}-1), 98.5 (C-6), 100.4 (C_{Rha}-1), 115.8 (C-5'), 116.2 (C-2'), 121.6 (C-6'), 133.1 (C-3).

Apigenin 7-*O*-rutinoside (**15**). Yellow powder. ESI-MS: *m/z* 577 $[M-H]^-$. UV (MeOH) λ_{max} nm: 268, 345; ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.08 (3H, *d*, *J* = 6.3, H_{Rha}-6), 3.18 (2H, *m*, H_{Glc}-4, H_{Rha}-4), 3.26-3.34 (2H, *m*, H_{Glc}-2, H_{Glc}-3), 3.46 (3H, *m*, H_{Rha}-3, H_{Rha}-5, H_{Glcb}-6), 3.59 (1H, *dd*, *J* =8.1, *J* = 7.7, H_{Glc}-5), 3.67 (1H, *dd*, *J* = 3.3, *J* = 1.3, H_{Rha}-2), 3.86 (1H, *d*, *J* = 11.1, H_{Glca}-6), 4.56 (1H, br *s*, H_{Rha}-1), 5.05 (1H, *d*, *J* = 7.0, H_{Glc}-1), 6.41 (1H, *brs*, H-6), 6.72 (2H, br *s*, H-3, H-8), 6.91 (2H, *d*, *J* = 8.5, H-3', H-5'), 7.88 (1H, *dd*, *J* = 8.2, H-2', H-6'). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra): δ 18.2 (C_{Rha}-6), 66.5 (C_{Glc}-6), 68.7 (C_{Rha}-5), 70.0 (C_{Glu}-4), 70.7 (C_{Rha}-2), 71.3 (C_{Rha}-3), 72.5 (C_{Rha}-4), 73.5 (C_{Glc}-2), 76.1 (C_{Glc}-5), 76.8 (C_{Glc}-3), 95.4 (C-8), 99.8 (C-6), 100.4 (C_{Glc}-1), 100.9 (C_{Rha}-1), 103.7 (C-3), 113.5 (C-3', C-5'), 128.3 (C-2', C-6').

3-*O*-*p*-Coumaroylquinic acid methyl ester (**17**). White powder. *m/z* 351 $[M-H]^-$. UV (MeOH) λ_{max} nm: 226, 300 (sh), 312; ^{1}H-NMR (CD_3OD, 500 MHz): coumaroyl moiety: δ 6.31 (1H, *d*, *J* = 15.9, H-8), 6.83 (2H, *d*, *J* = 8.7, H-3, H-5), 7.47 (2H, *d*, *J* = 8.7, H-2, H-6), 7.61 (1H, *d*, *J* = 15.9, H-7). quinic acid moiety: quinic acid moiety: δ 2.06 (2H, *dd*, *J* = 6.2, *J* = 13.6, H-6a), 2.14 (H, *m*, H-2a), 2.22 (2H, *m*, H-2b, H-6b), 3.72 (3H, *s*, $COO\underline{C}H_3$), 3.76 (1H, *dd*, *J* = 3.3, *J* = 8.0, H-4), 4.17 (1H, *dt*, *J* = 6.4, *J* = 3.2, H-3), 5.32 (1H, *dt*, *J* = 8.2, *J* = 4.2, H-3); ^{13}C-NMR (CD_3OD, extracted from 2D-HMBC and HSQC spectra): coumaroyl moiety: δ 115.1 (C-8), 116.7 (C-3, C-5), 125.6 (C-1), 131.0 (C-2, C-6), 146.8 (C-7), 161.2 (C-4), 167.2 (C-9); quinic acid moiety δ 38.0 (C-6), 38.2 (C-2), 51.5 ($-CO_2\underline{C}H_3$), 67.1 (C-5), 69.5 (C-4), 70.8 (C-3), 75.2 (C-1), 173.0 ($-\underline{C}O_2CH_3$).

Ibotanolide (**18**). White powder. ESI-MS: *m/z* 461 $[M-H]^-$. UV (MeOH) λ_{max} nm: 304 (sh), 325, 344(sh); ^{1}H-NMR (DMSO-d_6, 500 MHz): coumaroyl moiety : δ 6.40 (1H, *d*, *J* = 16.0, H-8), 6.81 (2H, *d*, *J* = 8.3, H-3, H-5), 7.54 (2H, *d*, *J* = 8.6, H-2, H-6), 7.58 (1H, *d*, *J* = 16.1, H-7); glucosyl moiety : δ 3.24 (1H, *m*, H-3), 3.28-3-36 (1H, *m*, H-2, H-4), 3.64 (1H, *dd*, *J* = 8.5, *J* = 6.5, H-5), 4.20 (1H, *dd*, *J* = 12.0, *J* = 7.0, H_b-6), 4.49 (1H, *dd*, *J* = 12.0, *J* = 2.0, H_a-6), 4.71 (1H, *d*, *J* = 7.0, H-1); hydroxytyrosol moiety: δ 2.56 (2H, *t*, *J* = 7, H–7), 3.50 (2H, *t*, *J* = 7, H-8), 6.68-6.73 (2H, *m*, H-2, H-6), 6.90 (1H, *m*, H-5). ^{13}C-NMR (DMSO-d_6, extracted from 2D-HMBC and HSQC spectra), coumaroyl moiety: δ 113.2 (C-8), 116.1 (C-3, C-5), 125.1 (C-1), 130.2 (C-2, C-6), 145.1 (C-7), 159.8 (C-4), 166.7 (C-9); hydroxytyrosol moiety: δ 38.3 (C-7), 62.1 (C-8), 116.8 (C-2), 117.8 (C-5), 123.1 (C-6), 130.7 (C-1), 144.9 (C-4).; glucosyl moiety: δ 63.9 (C-6), 70.5 (C-4), 73.5 (C-2), 74.2 (C-5), 76.1 (C-3), 102.7 (C-1).

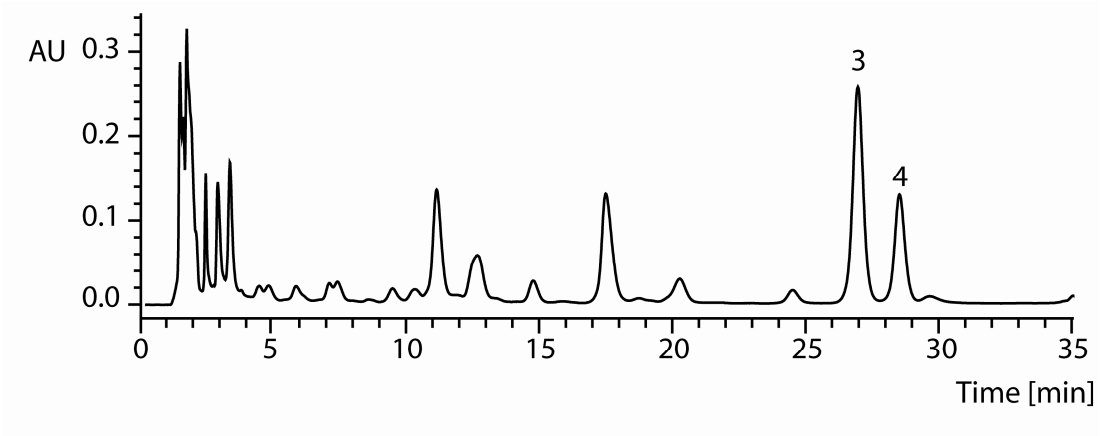
N_1, N_5, N_{10}, N_{14}-tetra-*p*-coumaroylspermine (**19**). White powder. ESI-MS: *m/z* 785 $[M-H]^-$ ^{1}H-NMR (CD_3OD, 500 MHz): 1.46-1.72 (4H, *m*, H-7, H-8), 1.74-1.94 (4H, *m*, H-3, H-12), 3.17-3.37 (4H, *m*, H-2, H-13), 3.36-3.57 (8H, *m*, H-4, H-6, H-9, H-11), 6.38 (2H, *d*, *J* = 15.5, H-8', H-8''''), 6.64-6.83 (10H, *m*, H-3', H-3'', H-3''', H-3'''', H-5', H-5'',H-5''', H-5'''', H-8'', H-8'''), 7.31-7.54 (12 H, *m*, H-2', H-2'', H-2''', H-2'''', H-6', H-6'', H-6''', H-6'''', H-7', H-7'', H-7''', H-7''''. ^{13}C-NMR (CD_3OD, extracted from 2D-HMBC and HSQC spectra): δ 25.6 (C-7)[a], 27.1 (C-8)[a], 28.2 (C-3)[b], 30.1 (C-12)[b], 37.4 (C-2, C-13), 45.2 (C-4) [a], 46.2 (C-11) [a], 46.5 (C-6) [b], 48.3 (C-9) [b], 114.1 (C-3'', C-3''', C-5'', C-5'''), 116.1 (C-3', C-3'''', C-5', C-5'''', C-8'', C-8'''), 117.8 (C-8', C-8''''), 129.9 (C-2', C-2'''', C-6', C-6''''), 130.1 (C-2'', C-2''', C-6'', C-6'''), 141.1 (C-7', C-7''''), 143.8 (C-7'', C-7'''), 160.6 (C-4', C-4'', C-4''', C-4''''), 169.3 (C-9', C-9'', C-9''', C-9'''').

Gingerglycolipid A (**20**). White solid. ESI-MS: *m/z* 721 $[M+HCOO^-$. ^{1}H-NMR (CD_3OD, 500 MHz): δ 0.97 (3H, *t*, *J* = 7.5, H-18'), 1.34 (8H, *m*, H-4', H-5', H-6', H-7'), 1.62 (2H, *m*, H-3'), 2.07 (2H, *m*, H-8'), 2.06 (2H, *m*, H-17'), 2.34 (2H, *t*, *J* = 7.4, H'-2), 2.81 (4H, *t*, *J* = 5.3, H-11', H-14'), 3.50 (2H, *m*, $H_{Gal'}$-3, $H_{Gal''}$-5), 3.68 (1H, *m*, H_b-3), 3.70 (1H, *m*, $H_{Galb'}$-6), 3.72 (3H, *m*, $H_{Gal'}$-5, $H_{Gal''}$-6), 3.75 (1H, *m*, $H_{Gal''}$-3),

3.80 (1H, *m*, $H_{Gal'}$-4), 3.83 (1H, *m*, H_a-3), 3.87-3.92 (4H, *m*, $H_{Gal'}$-2, $H_{Gal''}$-2, $H_{Gala'}$-6, $H_{Gal''}$-4), 3.99 (1H, *m*, H-2), 4.15 (2H, *m*, H-1), 4.26 (1H, *d*, *J* = 7.0, $H_{Gal'}$-1), 4.87 (1H, *d*, *J* = 3.5, $H_{Gal''}$-1), 5.31-5.40 (6H, *m*, H-9', H-10', H-12', H-13', H-15', H-16'). ^{13}C-NMR (CD_3OD, extracted from 2D-HMBC and HSQC spectra): δ 15.1 (C-18'), 22.0 (C-17'), 26.6 (C-3'), 27.0 (C-11', C-14'), 28.7 (C-8'), 30.8 (C-4', C-5', C-6', C-7'), 35.6 (C-2'), 63.4 ($C_{Gal''}$-6), 67.1 (C-1), 68.6 ($C_{Gal'}$-6), 70.3 (C-2), 70.8 ($C_{Gal'}$-4, $C_{Gal''}$-2), 71.7 ($C_{Gal''}$-4),72.1 ($C_{Gal''}$-3), 72.7 (C-3), 73.1 ($C_{Gal''}$-5), 73.2 ($C_{Gal'}$-2), 75.1 ($C_{Gal'}$-5), 75.3 ($C_{Gal'}$-3), 96.6 ($C_{Gal''}$-1), 101.3 ($C_{Gal'}$-1), 128.9 (C-10'), 129.7 (C-12', C-13', C-15'), 131.6 (C-9'), 133.4 (C-16'), 176.0 (C-1').

Data D4.

Quantification of linarin (3) and pectolinarin (4)



HPLC analyses were performed in triplicate on an Alliance 2695 instrument (Waters) equipped with a 996 PDA detector. Separations were performed on a SunFire C_{18} column (3.5 μm, 150 x 3.0 mm i.d., Waters) equipped with a guard column (10.0 x 3.0 mm i.d.). The flow rate was 0.5 mL/min. The mobile phase consisted of water with 0.05% formic acid (solvent A) and acetonitrile with 0.05% formic acid (solvent B). A gradient of 20 to 25% B in 30 min, and to 95% B in 10 min was used

3.4. Press and media

MAPs and their traditional use in Valais are of particular interest. This thesis has led to the writing of several articles in the German-speaking or French-speaking Swiss media and also inspired German-speaking televisions SF1 (Schweizer Fernsehen 1) and 3SAT to conduct a scientific report on wild food plants filmed in canton of Valais and in the University of Basel. In addition, the work has motivated the organizers of an economic forum to invite the author to a conference in the Casino of Saxon.

The author of this work has also designed with Prof. Dr. Hostettmann a nature trail in la Fouly (VS) on request of the local authorities to meet the expectations of many tourists in the region.

Die Heilkraft der Natur (29.08.2012)

Schweizer suchen nach pflanzlichen Wirkstoffen. Forscher suchen neuerdings mit Unterstützung der Schweizer Bergbevölkerung nach alten Pflanzenarten mit besonderen Inhaltsstoffen.
"Aufgrund ihres speziellen Standorts müssen diese Pflanzen schwierige Situationen wie Sonneneinstrahlung oder eisige Kälte überleben", erläutert der Basler Biologe Christian Abbet. Dazu haben sie zahlreiche Stoffe erzeugt, die sie schützen. "Ich bin überzeugt, dass die Alpenpflanzen für Kosmetika oder die Lebensmittelbranche und auch Pharmazie sehr interessant sind."

Link: http://www.3sat.de/page/?source=/nano/medizin/164377/index.html

Alpenkräuter für den Supermarkt (30.08.2012)

Alpenpflanzen müssen unter harten Bedingungen überleben. Forscher versuchen jetzt die Kraft der Alpenkräuter für den Menschen nutzbar zu machen, zum Beispiel in schmackhaften Salaten. Das Wissen um die wirksamsten Alpenkräuter sammeln sie in uralten Büchern und bei der Walliser Bergbevölkerung.

Link: http://www.srf.ch/sendungen/einstein/raketen-gegen-gewitter-unterwasser-baustelle-alpen-kraeuter

RAPUNZELBLÜTEN IN HASELNUSSÖL

Vorspeise für 4 Personen

3 Handvoll noch geschlossene Rapunzelblüten (Betonienblättrige und Ährige Rapunzel) 1 Minute über Dampf garen, auskühlen lassen und mit der Sauce mischen. Dazu Butterbrot servieren.

Sauce
1 Prise Kräutermeersalz
½ EL Zitronensaft
½ EL Sherry
1 EL Haselnussöl
1 EL mildes Sonnenblumenöl

Meret Bissegger, «Meine wilde Pflanzenküche», AT-Verlag, 49.90 Franken

FOTOS: SHUTTERSTOCK, SIFFERT/AT-VERLAG

ECHTE EDELRAUTE, ARTEMISIA UMBELLIFORMIS

Familie: Korbblütler (Asteraceae)
Habitat: Hochgebirge, 1300 bis 3700 Meter
Geschützt: Ja. Sammeln verboten
Anbau: Ja. Auf etwa 1 Hektar
Verwendung: Im Wallis und in Savoyen wird daraus ein aromatischer Likör gebraut – der Génépi. Weil einige Edelraute-Sorten recht viel vom Nervengift Thujon enthalten, das zu Wahnvorstellungen führen kann, wurde eine Thujon-freie Sorte gezüchtet. Der Maler Van Gogh soll sich übrigens im Thujon-Rausch das Ohr abgeschnitten haben.

KUGELIGE TEUFELSKRALLE, PHYTEUMA ORBICULARE

Familie: Glockenblumengewächse (Campanulaceae)
Habitat: Berg- und Magerwiesen, bis 2400 Meter
Geschützt: Nein
Anbau: Nein
Verwendung: Die Kugelige Teufelskralle, die auch den Namen Rundköpfiger Rapunzel trägt, wird von alten Walliser Bergvölkern als Salat zubereitet. Die Blüten können ebenfalls gegessen werden. Sie schmecken sehr süss. Auch von anderen Rapunzelarten, beispielsweise der Betonienblättrigen Rapunzel (Ph. betonicifolium) und der Ährigen Rapunzel (Ph. spicatum), ist bekannt, dass sie sehr schmackhaft sind (siehe Rezept).

ARNIKA, ARNICA MONTANA

Familie: Korbblütler (Asteraceae)
Habitat: 600 bis 2700 Meter
Geschützt: Nein
Anbau: Nein (in der Schweiz). Es gibt aber eine Sorte, die für den Feldanbau geeignet ist.
Verwendung: Verwendet werden die Blütenköpfchen. Sie enthalten verschiedene Stoffe (ätherische Öle, Flavonoide, Sesquiterpenlactone), die entzündungshemmend und antiseptisch wirken. Da auch toxische Wirkungen bekannt sind, kann Arnika nur äusserlich als Tinktur, Salbe oder Gel angewendet werden. Einsatzgebiete sind: Verletzungen und rheumatische Muskel- und Gelenkbeschwerden.

EDELWEISS, LEONTOPODIUM ALPINUM

Familie: Korbblütler (Asteraceae)
Habitat: 1800 bis 3000 Meter
Geschützt: Ja. Sammeln verboten
Anbau: Ja, etwa 1 Hektar
Verwendung: Bislang wurden vor allem die Edelweisssäuren verwendet. Sie stecken in vielen Kosmetikprodukten, sollen die Erbsubstanz schützen und so der Hautalterung vorbeugen. Innsbrucker Forscher haben jüngst aus den Wurzeln des Edelweiss einen neuen Stoff isoliert: Leoligin. Im Tierversuch zeigte sich, dass er der Verdickung von Gefässwänden und damit der Arteriosklerose entgegenwirken kann. Künftig könnte Edelweiss also auch zur Therapie von Gefässerkrankungen eingesetzt werden.

HEIDELBEERE, VACCINIUM MYRTILLUS

Familie: Heidekrautgewächse (Ericaceae)
Habitat: Ebene bis 2400 Meter
Geschützt: Nein
Anbau: Ja, etwa 45 Hektaren
Verwendung: Getrocknete Heidelbeeren sind als Durchfallmittel bekannt. Eine neue kleine Studie deutet darauf hin, dass sie auch bei Gedächtnisproblemen helfen könnten. Neun 70-jährige Probanden, die an verfrühten Gedächtnisstörungen litten, tranken zwölf Wochen lang täglich zwei Gläser Heidelbeersaft. In anschliessenden Gedächtnistests schnitt diese Gruppe viel besser ab als eine Vergleichsgruppe, die einen anderen Saft getrunken hatte.

GELBER ENZIAN, GENTIANA LUTEA

Familie: Enziangewächse (Gentianaceae)
Habitat: 750 bis 2500 Meter
Geschützt: Nein
Anbau: Nein. Wildsammlungen im Jura
Verwendung: Die Wirkstoffe stecken in der Wurzel. Der Gelbe Enzian ist vor allem für seine Bitterstoffe bekannt (Gentiopicrin, Amarogentin). Sie schützen die Pflanze vor Tierfrass. Beim Menschen sollen sie die Sekretion von Magen- und Gallensaft und die Verdauung wie den Appetit anregen. Aus dem Wurzelextrakt werden Tees, Tinkturen oder Bitterliköre (Magenbitter) hergestellt. In den USA gibt es mit Moxie sogar einen Softdrink, in dem Enzian steckt.

ALPENROSE, RHODODENDRON FERRUGINEUM

Familie: Heidekrautgewächse (Ericaceae)
Habitat: 500 bis 2800 Meter
Geschützt: Nein
Anbau: Nein. Wildsammlungen in Bergün
Verwendung: Die Alpenrose ist ein grosser Strauch, der bis zu 100 Jahre alt werden kann. Dank einer vielfältigen Mischung aus Polyphenolen ist die Pflanze für extreme Wachstumsbedingungen in der Höhe gerüstet – Temperaturwechsel, hohe UV-Strahlung, Trockenheit. Auf diese Polyphenole hat es die Kosmetikfirma Mibelle Biochemistry abgesehen. Die Substanzen sollen auch die menschliche Haut schützen. Das Alpenrosenextrakt, isoliert aus den Blättern, steckt bereits in einigen Kosmetikprodukten.

FLORA ALPINA

Ethnobotaniker und Pharmakologen suchen nach alten Alpenpflanzen, um neue Wirkstoffe zu finden

VON SABINE OLFF (TEXT) UND BIRGIT LANG (ILLUSTRATIONEN)

Auf rund 10 000 Quadratmeter Anbaufläche gedeiht in den Schweizer Bergen die berühmteste aller Alpenblumen: das Edelweiss. Kosmetikfirmen sind die Hauptabnehmer. Sie isolieren daraus ein Extrakt, das die Haut vor Alterung schützen soll. Es steckt bereits in vielen Cremetiegeln.

Das Edelweiss ist wie viele andere Bergpflanzen mit besonderen Inhaltsstoffen ausstaffiert. Weil das Klima in hohen Lagen rau, die UV-Strahlung hoch und die Böden karg sind, synthetisieren die Pflanzen andere Substanzen als ihre Verwandten im Tal. Das macht sie für die Kosmetik-, Pharma- und Nahrungsmittelbranche interessant. Edelweiss, Enzian und Arnika werden längst entsprechend genutzt.

«Ein grosses Potenzial liegt aber noch brach», sagt Christoph Carlen, Leiter des Forschungsdepartements von Agroscope Changins-Wädenswil (ACW) in Conthey. ACW hat deshalb kürzlich zum ersten internationalen Symposium zu Medizinal- und Gewürzpflanzen aus dem Berggebiet nach Saas-Fee geladen. Es ging um neue Entdeckungen, Anwendungen und Kultursorten.

Allein in den Alpen gibt es etwa 4500 Pflanzenarten. «Nur ein Bruchteil davon ist genau beschrieben», sagt Carlen

Die Fahndung nach interessanten Pflanzen gleicht der Suche nach der Stecknadel im Heuhaufen. Zunächst muss man eine Fährte haben. Diego Rivera, Ethnobotaniker an der Universität Murcia (Spanien), klappert dafür sechs traditionsreiche, isolierte Alpenvölker ab, etwa die Walser in Bosco Gurin. Er will herausfinden, welche Pflanzen die Alpenbewohner früher als Medizin oder als Salat genutzt haben. Dafür wühlt er sich durch alte Literatur, Lexika und Kochbücher und befragt die greise Bevölkerung.

Ähnlich arbeitet eine Gruppe von Forschern der Abteilung für pharmazeutische Biologie an der Universität Basel. Sie suchen nach vergessenen Nahrungspflanzen, analysieren ihre Inhaltsstoffe und wollen langfristig die besonders gesunden Sorten anbauen.

Christian Abbet ist dafür in seinen Heimatort Orsières im Wallis gereist und hat 20 Einheimische interviewt. Der älteste war 97 Jahre alt. 107 essbare Wildpflanzen hat Abbet so identifiziert; rund 30 besonders schmackhafte Arten sollen nun analysiert werden.

Die Ziegenhirten assen die Blüten der Teufelskralle

Beispielsweise die Alpenkratzdistel – ein Snack der Schäfer. Der Name tönt wie die Pflanze ist: ziemlich stachelig. Gegessen wird der innere Teil des Blütenkelchs. «Er ähnelt einer Artischocke», sagt Abbet, der alle Gewächse selbst probiert hat. Auch die Erdkastanie. Die kugelige Knolle schmeckt wie eine Marroni. Sie sei früher während der Fastenzeit gegessen worden. Abbet warnt jedoch vor dem Sammeln: «Es gibt viele ähnliche Pflanzen, die sehr giftig sind.»

Eine weitere Wiederentdeckung ist die Kugelige Teufelskralle. Die Einheimischen essen die jungen Blätter als Salat. Und die Ziegenhirten kauten früher die sehr süss schmeckenden Blüten. Die Teufelskralle ist die erste Pflanze, die Abbet und seine Kollegen jüngst im Chemielabor zu analysieren begannen. Und sie wurden prompt überrascht: Sie entdeckten zwei völlig neue Substanzen aus der Gruppe der Saponine. Derzeit wird in biologischen Tests geklärt, welche Wirkungen sie haben.

In Saas-Fee war die Teufelskralle bereits Gesprächsstoff. Ihr Name kursierte auch schon bei Ricola. Der Bonbonhersteller, der all seine Kräuter in den Schweizer Bergen anbauen lässt, hält ständig Ausschau nach neuen alten Pflanzen, die gut schmecken und bei Husten und Heiserkeit helfen können. Input holt sich Ricola von Kräuterbauern sowie von Agroscope ACW. Über neuste Projekte herrscht jedoch Stillschweigen.

Auch bei Mibelle Biochemistry, die Kosmetikfirmen mit Pflanzenextrakten beliefert und die Alpenflora in einem Schwerpunktprogramm erforscht, gibt man sich zugeknöpft. Mibelle-Forscher Frank Gafner sagt: «Alpenpflanzen sind nicht nur wissenschaftlich interessant, sondern auch weil sie sich gut vermarkten lassen.» Man assoziiere sie mit Reinheit, Swissness, Exklusivität.

Ihr neustes Produkt ist ein Extrakt aus den Blättern der Alpenrose. Es enthält Polyphenole, die antioxidativ wirksam sind und vor Alterungsprozessen schützen sollen, sowie Stoffe, die Viren und Bakterien in Schach halten. Das Blattmaterial wird derzeit von Biobauern in Bergün gesammelt. «Der Anbau erweist sich als ziemliche Knacknuss», sagt Gafner.

Die beste Rosenwurz stammt aus dem Walliser Saastal

Die Anbaufähigkeit ist bei der Suche nach neuen alten Arten ein wichtiges Kriterium. Schliesslich gewährleisten die Kulturpflanzen einen guten Ertrag sowie eine konstante Menge an Inhaltsstoffen; und sie wirken dem Sammeln von Wildpflanzen entgegen.

Agroscope ACW hat in den vergangenen Jahren bereits einige Alpenkulturpflanzen auf den Markt gebracht, etwa Edelweiss, Schafgarbe, Frauenmantel und die Echte Edelraute (Génépi). Der jüngste Zuchterfolg ist die Rosenwurz. 2003 weckte ein Artikel in der Zeitschrift «Newsweek» das Interesse der ACW-Forscher. Darin wurden die stresslindernden Eigenschaften der Pflanze beschrieben. Bereits in der Antike wurde sie entsprechend genutzt; in Russland und Skandinavien ist sie fester Bestandteil der Volksmedizin.

Hierzulande geriet die Pflanze hingegen in Vergessenheit. Dabei wächst sie auch in den Schweizer Bergen. Die hiesige Rosenwurz ist sogar reicher an den gewünschten Inhaltsstoffen (Salidrosid und Rosavin) als die russischen und skandinavischen Ökotypen. Am robustesten und ertragreichsten erwiesen sich Pflanzen aus den Hängen um den Mattmarkdamm im Saastal. Die besten unter ihnen haben die Forscher zur Züchtung der Sorte Mattmark ausgewählt. Erste Kulturen wurden auf 2000 Quadratmetern im Berggebiet angelegt.

AUSFLUGSTIPPS

► Alpenflora-Erlebnispfad bei Savognin
Von der Bergstation Somtgant (2112 m) aus wandert man in gut zwei Stunden über Mot Laritg und Monas nach Tigignas (1600 m). Die Wanderung führt an rund 100 verschiedenen Alpenblumen, Heilpflanzen und Alpenkräutern vorbei, die alle speziell gekennzeichnet sind. Infos: www.savognin.ch

► Alpinum Schatzalp bei Davos
Das Alpinum ist ein privater botanischer Garten. Insgesamt können rund fünf Hektaren Gartenanlage durchwandert werden. Zu entdecken gibt es 3500 Pflanzen aus allen Gebirgen der Welt. Infos: www.alpinum.ch

In der Ausstellung «Edelweiss – Mythos und Paradox» zeigen die Botanischen Gärten von Genf und Zürich alles Wissenswerte über die Schweizer Symbolpflanze. Bis 16. Oktober 2011, www.expo-edelweiss.ch

DIE VIELEN NAMEN DES EDELWEISS:
Löwenfüsschen, Wollblume, Stella alpina (Stern der Alpen), Alv etérn (Ewigweiss), Bauchwehblüml

ROSENWURZ, RHODIOLA ROSEA

Familie: Dickblattgewächse (Crassulaceae)
Habitat: 1000 bis 3000 Meter
Geschützt: Nein.
Anbau: Ja, erste Kulturen angelegt
Verwendung: Nach vier Jahren ist die Rosenwurz reif für die Ernte. Die begehrten Inhaltsstoffe (v. a. Rosavin, Tyrosol, Salidrosid) stecken in der Wurzel. Um Stress zu lindern und die körperliche wie die geistige Leistungsfähigkeit zu steigern, haben schon russische Kosmonauten das Wurzelextrakt genutzt. Und selbst Spitzensportler setzen auf die Pflanze. Seit letztem Jahr ist in der Schweiz mit Vitango das erste Rosenwurzpräparat als Antistressmittel zugelassen. Im Februar trugen britische Experten alle qualitativ hochwertigen Studien zur Rhodiola zusammen und kamen zu dem Schluss, dass das Extrakt mental wie physisch wirksam sein könnte. Sie bemängeln jedoch, dass einzelne Studien noch nicht von unabhängigen Forschern repliziert worden sind.

Date: 27.08.2012

SCHWEIZER ILLUSTRIERTE

Ringier AG
8008 Zürich
044/ 259 63 63
www.schweizer-illustrierte.ch

Genre de média: Médias imprimés
Type de média: Magazines populaires
Tirage: 201'458
Parution: hebdomadaire

N° de thème: 278.15
N° d'abonnement: 1085976
Page: 80
Surface: 93'938 mm²

KEINE LANGEWEILE Der extra für Kinder angelegte Murmeltierweg ob Verbier VS bietet mehr als schöne Landschaft.

Besuch bei den Murmeltieren

Auf dem Lehrpfad bei La Fouly im Val Ferret verläuft der Themenweg «Charlotte la Marmotte» oder auf gut Deutsch: CHARLOTTE DAS MURMELTIER. Hier können Kinder auf den Spuren der «Mungge» wandern – und dazu spielerisch lernen. Eine Freude für Jung und Alt.

Auf dem speziell für Kinder errichteten Lehrpfad ist Charlotte, das drollige Murmeltier, ständige Begleiterin. Ist man aufmerksam, kann man Murmeli beobachten. Auf dem Pfad stehen zehn Tafeln mit Fragen, die es zu beantworten gilt. Die Lösung der Aufgaben bereitet kaum grosses Kopfzerbrechen, gut beobachtet ist schon halb gewonnen. Beispiel gefällig? In der herrlichen Gebirgslandschaft glitzert im Hintergrund das Eis des A-Neuve-Gletschers. Ein Schwarz-Weiss-Foto von 1930 auf der Postentafel zeigt denselben Eisstrom. «Schau dir den Gletscher an, und vergleiche ihn mit dem Foto von 1930. Was fällt dir auf?», steht da. Oder: Ein Foto zeigt eine Scheune, Baujahr 1900. Neben der Fotografie sind verschiedene Werkzeuge abgebildet. Darunter die Gretchenfrage: «Einige davon gab es damals noch nicht – welche?» An einem weiteren Posten können Rufe von Tieren abgehört werden, die sodann dem (möglichst zutreffenden) Piktogramm zugeord-

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net werden müssen. Im Gepäck sollte zwingend ein Bleistift mitgeführt werden. Für den ausgeschilderten und mühelos zu begehenden Rundweg benötigt man etwa zwei Stunden. Am Ziel können die Antworten überprüft werden.

Auf derselben Route wie der Lehrpfad von Charlotte dem Murmeltier verläuft auch ein botanischer Lehrpfad. Hier werden Erwachsene in die Geheimnisse der Natur eingeweiht. «Nicht alles, was natürlich ist, ist auch gut»: Christian Abbet muss es wissen. Er ist Apotheker und Doktorand in Pharmazeutischer Biologie an der Universität Basel. Zusammen mit Professor Kurt Hostettmann hat er den botanischen Lehrpfad eingerichtet. «Wir haben uns für zwanzig Pflanzen entschieden, die in der Region gedeihen», erklärt Christian Abbet. Dabei ist so ziemlich alles, was in der Kräuterlehre Rang und Namen hat. «Fehlende Kenntnisse bei der Verwendung von Pflanzen führen leider immer wieder zu Vergiftungen. Meistens sind Kinder betroffen. Darum scheint mir wichtig, dass auch Kinder diesen Pfad gehen», sagt Abbet.

▶ **WEITERE INFORMATIONEN** www.verbier.ch

SCHNÜGEL Murmeltiere sind drollig und begeistern Kinder und Erwachsene gleichermassen.

FAMILIENFERIEN TOTAL

Spiel, Spass und Staunen für Gross und Klein

▶ **FERIENDORF FIESCH** Traditionelle Walliser Ferienchalets, komfortable Pavillons, ein Bett in der 2011 neu eröffneten Jugendherberge oder ein modernes Berghaus? Im Sport- und Ferienresort Fiesch ist all das zu haben. In den romantischen Chalets finden hier traditionsbewusste Familien ebenso Entspannung wie unkomplizierte Winter- und Sommersport-Fans. Und hier steigen auch Individualgäste auf der Durchfahrt (ganz besonders auf zwei Rädern) gerne ab. Das Motto: «All inclusive» – alles ist dabei! www.sport-ferienresort.ch

▶ **AQUAPARC** Der Wasserpark in Le Bouveret ist Adrenalin pur: rasante Rutschpartien mit einer Länge von insgesamt einem Kilometer! www.aquaparc.ch/de

▶ **BRIGERBAD** Das grösste Freiluft-Thermalbad der Schweiz! Brigerbad samt grossem Camping und weiteren Übernachtungsmöglichkeiten ist von überall aus der Region innert weniger Minuten erreichbar. Auf Kinder und Abenteurer wartet eine 182 Meter lange Rutschbahn! www.brig-belalp.ch

▶ **SWISS VAPEUR PARC** Nur einige Schritte vom Ufer des Genfersees entfernt liegt das 17 000 Quadratmeter grosse Gelände. Miniatureisenbahnen, auf denen man fahren kann, schlängeln sich durch die Schweiz im Kleinformat. Die Eisenbahn-Schweiz als Traumland. www.swissvapeur.ch

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KINDERPARADIES IN BELLWALD

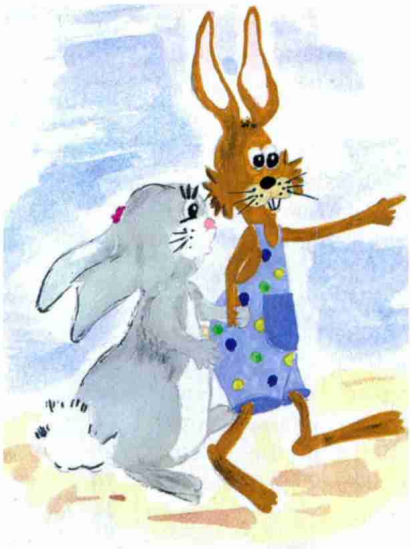
Von Zwergen, Schneewittchen und Hasenliebe

► **BELLWALD** Im Sommer bietet Bellwald im Oberwallis die ideale Infrastruktur für Familien mit Kindern. Moderne Spielplätze, idyllische Grillstellen, Tipi-Zelt, Bergseen und sogar kinderwagentaugliche Wanderwege stehen den Gästen zur Verfügung. Die vielfältigen Animationsprogramme reichen von betreuten Kinderabenteuerwochen über Spielnachmittage und Spurensuche bis zu Indianertreffs. Wasserspiele, Badeplausch, Tennisturniere und Kletterpartien – in Bellwald haben Kinder noch Platz zum Austoben.

► **MÄRLIWEG** Mit Schneewittchen und den sieben Zwergen wandern? Elfen und Feen zwischen den Bäumen entdecken? Auf dem Bellwalder Märliweg ist vieles möglich. Die fantasievolle Wanderung beginnt bei den Sportanlagen. Dort können die Besucher lesend in die erste Geschichte eintauchen. Dem Alpweg entlang geht es dann weiter bis zur Mittelstation der Alp Richenen. Für die acht Märchen und den sechs Kilometer langen Weg benötigt man knapp zwei Stunden.

► **HASENLIEBE** Mitte September 2012 startet der Märchenweg «Hasenliebe»: Auf neun interaktiven Stationen, unter anderem «Forschungsstation» oder «Baumhaus», wird die Geschichte von Bella, die Waldi besucht, erzählt. Sie soll vermitteln, wie wichtig die Nächstenliebe untereinander ist.

Die verschiedenen Stationen wurden in die Geschichte eingebunden und inhaltlich mit der Tier- und Pflanzenwelt und mit den Aktivitäten in Bellwald verknüpft. Bei allen Stationen können die Besucher durch Spiele und Aktivitäten selber aktiv werden.
Weitere Infos: www.bellwald.ch



BELLA UND WALDI Hase Waldi zeigt Häsin Bella die Natur und nimmt dabei die Besucher mit auf den Weg.

LE NOUVELLISTE SAMEDI 7 JUILLET 2012
jcz - jh

LA FOULY Avec «Charlotte la Marmotte», un sentier didactique permet d'aller à la découverte du Val Ferret.

Elle m'a dit d'aller siffler là-haut...

ALBANE BOCHATAY

Il se dégage un air d'authenticité dans la paisible station de la Fouly. Pourtant, l'heure n'était pas à l'hibernation ce dimanche 1er juillet. Le parcours de «Charlotte la Marmotte» a ouvert ses portes. Dans son sillage, dix postes mêlant aventure et réflexion. Ce nouveau sentier didactique reflète un tourisme doux qui respecte la nature. Les familles peuvent ainsi partir à la découverte des richesses du val Ferret. C'est Anne Zeller, animatrice socioculturelle au CREPA (Centre régional d'études des populations alpines), qui a pensé et développé le concept. La commune d'Orsières a lancé le mandat et apporté son soutien. «*Le but était de mettre en valeur le patrimoine de la Fouly*», confie l'animatrice. Un concept imaginatif: «Charlotte la Marmotte» poursuit un rêve, partir à l'aventure, baluchon sur le dos, invitant les enfants à la suivre sur un parcours d'environ deux heures.

Sur le sentier didactique «Charlotte la Marmotte», une dizaine de postes ont été créés, véritable jeu de piste qui fait le bonheur non seulement des enfants, mais aussi des adultes. MAMIN

A l'aventure...

Départ à l'office du tourisme de la Fouly. Un chemin facile nous attend à travers différents biotopes: forêt, torrents gorgés d'eau, glacier... Le premier poste propose un retour dans le passé. Un cliché des années trente est à comparer avec une jolie vue sur la station. Nous continuons notre chemin, au son du criquet, celui des Iscles peut-être? Un des postes est dédié à cette curiosité nationale de la Combe des Fonds. «*Il est très rare*», note notre guide. Bientôt apparaissent des roches calcaires, dont les secrets sont délivrés au poste 4, auquel le géologue Candide Gabioud a contribué. Plus loin, nous traversons la passerelle en savourant la vue sur le glacier de l'A Neuve. Au poste 9, l'activité coup de cœur de la créatrice du projet: différencier les cris des animaux de la vallée de Ferret. A nous le brâme du cerf et le sifflement de la marmotte. A la fin du parcours, les petits aventuriers débouchent sur le sentier suspendu et découvrent les réponses. Anne Zeller livre, ravie: «*C'est le paradis des enfants. L'âge idéal se situe entre 7 et 12 ans, mais chacun y trouve son compte*».

La responsable du projet a su collaborer avec les acteurs locaux: «*Ce fut avant tout une réflexion à plusieurs. L'équipe communale, notamment Nicolas Maillard, ainsi que les Forces Motrices d'Orsières ont fait un travail remarquable. J'ai entre autres reçu l'aide d'une collégienne, d'un retraité, d'un collectionneur de photos...*» Un projet qui rassemble trois générations, et qui promet de durer: le voyage de Charlotte sera prolongé jusqu'au Sentier des champignons, qui relie Orsières à Champex. «*Qui sait, bientôt naîtra peut-être Philemon le saumon?*», livre-t-elle, l'imagination débordante.

www.crepa.ch/charlotte-la-marmotte.

VINGT PANNEAUX DÉVOILENT LES VERTUS DE LA FLORE MÉDICINALE

Et pour celles et ceux qui souhaitent profiter pleinement de leur séjour à la Fouly, la station propose également de partir à la découverte des plantes alpines. Sur le même parcours que «Charlotte la Marmotte» a été inauguré le Sentier des plantes médicinales, parcours didactique composé de 20 panneaux explicatifs illustrés. Egalement initié par la commune d'Orsières, ce projet bénéficie de la collaboration de deux spécialistes des plantes thérapeutiques: Christian Abbet, pharmacien, et Kurt Hostettmann, professeur honoraire aux Universités de Lausanne et de Genève. Le microclimat de la station permet d'entrevoir une large palette d'espèces végétales. Une bonne opportunité de découvrir ou redécouvrir nos amies de la flore et d'en savoir davantage sur leurs propriétés thérapeutiques, voire toxiques. Pour les plus passionnés, les deux spécialistes se portent volontaires pour des visites guidées. Les réservations se font à l'Office du tourisme de la Fouly. Idéal pour observer l'orchidée et l'edelweiss sous un autre angle... Informations en composant le 027 783 27 17.

ROLAND CLERC

GALERIE PHOTOS+

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COLLOQUE AU JARDIN ALPIN DE CHAMPEX

«L'arolle, tout là-haut»

Le Jardin botanique alpin Flore-Alpe de Champex-Lac organise, les 13 et 14 juillet prochains, un colloque tout public et une excursion à Orsières sur le thème de l'arole. Les caractéristiques et les particularités biologiques et écologiques du pin des Alpes seront présentées au travers de six conférences à la portée du grand public.

La journée du vendredi se terminera par une visite libre du Jardin alpin, suivie d'un apéritif et d'un repas à Champex-Lac. Samedi 14 juin, c'est une excursion dans le Val d'Arpette qui permettra aux participants de se familiariser avec «L'arolle et la limite supérieure de la forêt.»

A l'exemple de ce colloque, les activités 2012 du Jardin Flore-Alpe évolueront principalement autour du thème de l'arolle. Plusieurs événements sont ainsi mis sur pied pour mettre en valeur cet exceptionnel conifère ainsi que ses nombreux usages, ceci en lien avec les recherches effectuées par le Centre alpin de phytogéographie (CAP). **PG/C**

Infos sur www.flore-alpe.ch

MÉMENTO

SALVAN

Autour du Moulin Fine. Du 7 juillet au 5 août, exposition de pastels de Françoise Gross sur le thème «Autour du Moulin Fine» à la galerie des Combles. Expo ouverte du vendredi au dimanche de 16 h à 19 h. Ce dimanche à 17 h, concert de flamenco avec le Trio Nuevo.

MARTIGNY

Lecteurs complices. Les Lecteurs Complices vous attendent le lundi 9 juillet (avec comme thème les romans policiers), le lundi 6 août (coups de cœur) et le mardi 11 septembre (l'Inde). Rendez-vous à chaque fois dès 16 h, à la Médiathèque de Martigny.

FULLY La brochure «Informations touristiques» est sortie de presse.

Une première pour Fully Tourisme

C'est une première. Fully Tourisme dispose désormais d'une brochure d'informations touristiques fort complète qui permet de faire le lien entre tous les acteurs et les partenaires du tourisme, se réjouit son directeur Eric Hamon: «*Pour Fully Tourisme et ses partenaires, il s'agit d'un nouvel outil de travail performant et pratique qui remplace une multitude de petits fascicules devenus vieillots et surtout incomplets*».

Cette brochure vise trois objectifs principaux: affirmer l'identité touristique de Fully; mettre en valeur le tourisme doux, ce qui se retrouve autant dans l'offre présentée que dans la ligne graphique de la brochure; répondre aux objectifs de la politique touristique définie en 2011 par la commune et la SD de Fully.

Devant leur stand mobile, les collaborateurs de Fully Tourisme (de gauche à droite, Anne-Laure Luisier, Eric Hamon, Laetitia Dorsaz et Daniela Padeste) présentent leur nouvelle brochure touristique. DR

Les produits-phares

La brochure met évidemment en exergue les quatre produits-phares de Fully, soit les produits du terroir et la viticulture, la châtaigne et son incontournable fête, la nature et la fameuse réserve naturelle des Follatères, les balades, douces ou sportives, tant en plaine que sur les hauts de Fully. Un cinquième volet est consacré à la culture (Belle Usine, expositions, patrimoine.)

Dans un souci de cohérence au niveau de la promotion touristique et de la communication, les quatre produits-phares sont aussi déclinés sur des panneaux, exposés notamment dans le nouveau stand mobile de l'OT.

La brochure comprend enfin un dépliant mobile, réédité chaque année, avec la liste des manifestations et une brève description, carte à l'appui, des partenaires du tourisme fulliérain.

En résumé, il s'agit d'un outil de synergie, annonce Eric Hamon: «*Cette brochure, c'est un lien physique entre tous les acteurs du tourisme, sur le papier pour la première fois. Il sera suivi de liens concrets dans le terrain, à l'exemple de la Corbeille du terroir qui sera lancée cet automne. Et d'autres projets sont en préparation.*»

En trois langues, la nouvelle brochure a été présentée en primeur lors de Fully en Terrasses et a suscité de très bons échos: «*Les réactions ont été positives autant de la part des touristes, qui découvrent l'étendue de notre offre, que des Fulliérains, qui découvrent eux aussi de nouvelles choses.*» Diffusée dans les offices du tourisme valaisans, elle est à disposition des commerçants et des privés à l'OT de Fully. **OLIVIER RAUSIS**

BAUME À TOUT FAIRE

DR

SOS PEAUX SÈCHES Un compagnon à porter en permanence sur soi vu qu'il est multitâche. Il soigne les zones sèches, hydrate les lèvres, soulage les gerçures et apaise les coups de soleil. De plus, il est 100% sans pétrole.
Superbaume, 22 francs chez Lush

DR

T'AS LA FRITE!

APPLICATION Développé par Cathy Yersin et Cedric Bosson, deux créatifs vaudois, le jeu pour iPad «Petite frite» fait un carton auprès des tout-petits. Ces derniers pourront jouer au Memory, recomposer des puzzles animés, créer leur propre théâtre musical ou encore décorer un paysage. Les parents n'ont même pas besoin de rester derrière, car tout y est intuitif, sans texte ni pub cachée.
«Petite frite», sur iPad dès 2 ans, 2 fr. 99 sur l'App Store

LES PLANTES QUI SOIGNENT ONT LE VENT EN POUPE

SANTÉ La Fouly (VS) inaugure dimanche son Sentier des plantes médicinales. Une superbe balade bourrée d'informations.

«La santé par les plantes, ça intéresse tout le monde. D'ailleurs 40% des médicaments en vente libre sont d'origine naturelle, explique le professeur Kurt Hostettmann, spécialiste des plantes et de phytochimie. Le problème, c'est qu'on a tendance à croire que tout ce qui est naturel est bon, alors que c'est faux. Les poisons les plus forts du monde sont eux aussi d'origine naturelle.» C'est en partant de ce constat que le professeur, accompagné du Dr Christian Abbet, a mis sur pied le Sentier des plantes médicinales et toxiques à La Fouly (VS).

Cette balade facile de 1,5 km, facilement réalisable en famille et jalonnée de vingt panneaux explicatifs, sera inaugurée dimanche. Les deux scientifiques à l'initiative du projet effectueront à cette occasion des tours guidés, activités qui seront proposées de nouveau tout au long de l'été. Au fil des pas, on découvre des orchidées, des alchémilles ou la reine-des-prés, une grande plante aux fleurs blanches qui fait baisser la fièvre. «Nous avons choisi 20 plantes représentatives de la région, mais nous pourrions en mettre beaucoup plus, s'enthousiasme le professeur Hostettmann. Lors des promenades guidées, nous aurons l'occasion de nous arrêter pour en décrire d'autres.»

Attention aux effets secondaires

La création de ce sentier s'inscrit pile dans la tendance des soins faits maison. Ces dernières années, de nombreux espaces ont ainsi été créés dans l'optique de présenter au public la flore médicinale d'une région. Le Jardin botanique de Genève consacre, par exemple, une partie de ses serres aux plantes qui soignent. La Fondation Gentiana, située à Leysin, est un espace unique au monde où les végétaux sont classés par utilisation thérapeutique. Pour le sentier de La Fouly, Kurt Hostettmann et Christian Abbet ont voulu mettre l'accent sur les éventuelles interactions et effets secondaires que peuvent avoir les plantes. Ils ont aussi mis en avant quelques spécimens toxiques, comme l'aconit, aux magnifiques fleurs violettes. «En Suisse, on recense 3000 intoxications par an à cause des plantes», explique Christian Abbet, pharmacien. «Le risque de confusion est grand, renchérit l'intarissable passionné Kurt Hostettmann. Il ne faut pas oublier que les plantes ne sont pas toutes comestibles.»

● **SANDRA IMSAND**
sandra.imsand@lematin.ch

La Fouly en fête, dimanche 1er juillet dès 9 h, parcours VTT, présentations et tours guidés du Sentier des plantes médicinales et de Charlotte la Marmotte. Animations.
www.orsieres.ch

Laurent de Senarclens

«Le risque de confusion entre les plantes est grand»
Professeur Kurt Hostettmann, spécialiste des plantes

Kurt Hostettmann (à g.) et Christian Abbet, créateurs du sentier, proposeront des visites guidées dimanche.

Christian Constantin a enthousiasmé les auditeurs du Forum de Saxon par son franc-parler et l'étendue de son expérience. Au second plan, Marie Pedroni, membre de la promotion économique de Saxon, animatrice de la soirée. BITTEL

ÉCONOMIE Succès pour la cinquième édition du Forum de Saxon.

Sous le signe du défi

PIERRE MAYORAZ

Salle comble à nouveau pour la cinquième édition du Forum économique de Saxon. Nombre de représentants de l'économie cantonale et plusieurs hommes politiques avaient fait le déplacement du casino de la cité de l'abricot pour prendre part à ce désormais incontournable rendez-vous de l'esprit d'entreprise dans notre canton.

Les organisateurs avaient placé la soirée 2011 sous le thème général du défi. Les trois orateurs l'ont abordé sous divers angles, chacun avec son expérience et sa sensibilité.

Le défi quotidien

Le sportif-entrepreneur Christian Constantin a ouvert les feux en évoquant sa double casquette de président du FC Sion et de patron d'un bureau d'architectes. Il a souligné les avantages qu'il pouvait obtenir en conjuguant les deux activités: *«L'image du FC Sion, excellente sur le plan romand, voire national, se reflète sur le travail de mon bureau. Les gains que ce dernier réalise me permettent de financer le club. Mais, il faut jouer serré et relever le défi chaque jour en donnant beaucoup de soi-même.»* Et Christian Constantin peut se poser en professionnel des défis lui qui ose affronter sans trembler les plus hautes autorités du football *«pour qu'il n'y ait qu'une seule justice»*, précise-t-il.

Le président du FC Sion tire un parallèle entre bâtir une équipe et construire un immeuble: *«Il faut tenir compte du facteur humain, respecter l'autre et les engagements pris. Il faut aussi regarder vers l'avenir, toujours avoir un coup d'avance.»* Les succès de l'homme d'affaires martignerain plaident en faveur de la méthode.

Le défi de la dette des Etats

A la faconde de Christian Constantin a succédé le sérieux d'Attilio Zanetti, directeur de l'unité d'analyse économique de la Banque nationale suisse. Selon lui, le temps presse: *«Il faut commencer maintenant à mettre en place les mesures qui doivent enrayer le cercle vicieux initié par la crise des «subprimes» et qui voit les dettes des Etats croître de façon exponentielle mettant à mal la confiance des ménages, le système bancaire, freinant les entrées fiscales et tuant la croissance qui seule permettrait de s'en sortir.»*

Son exposé a regroupé les nouvelles macroéconomiques qui nous parviennent par bribes en un tableau clair de la situation mondiale qui fait froid dans le dos et qui offre peu de portes de sortie.

Le défi de la recherche

Dernier orateur, Christian Abbet, chercheur en pharmacie à l'Université de Bâle, a expliqué comment sa passion d'enfant pour les plantes s'est transformée en un métier passionnant. Actuellement, il étudie les plantes comestibles oubliées des Alpes. 107 d'entre elles pousseraient en Valais. Le jeune chercheur a décrit les processus scientifiques de sa recherche tout en relevant la profonde connaissance botanique des gens de nos vallées qu'il a interrogés pour étayer certaines découvertes. Selon lui, la combinaison des traditions valaisannes couplée à la pureté que symbolisent les Alpes constitue un excellent atout marketing, un défi à relever pour le Valais.

PHARMAZEUTISCHE BIOLOGIE

Hightechsuche nach Naturstoffen

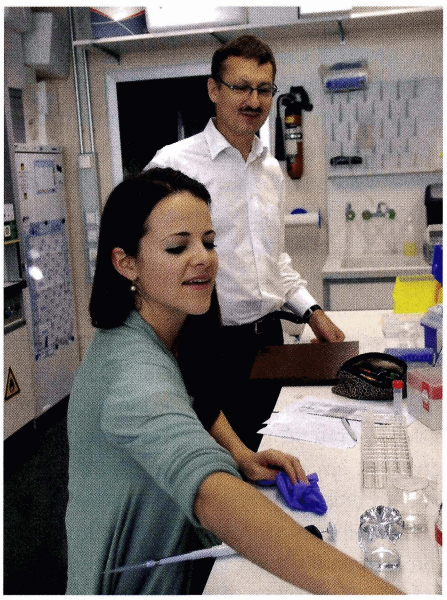
Eine Hochleistungstechnologieplattform im Rücken, suchen Naturstoffforscher der Universität Basel in pflanzlichen Extrakten nach bioaktiven Molekülen, deren Strukturen die Entwicklung pharmazeutischer Wirkstoffe für neue Therapieansätze anleiten können. Daneben sind vergessene gesundheitsfördernde Nahrungspflanzen ein Forschungsthema.

BEATE PEISELER-SUTTER

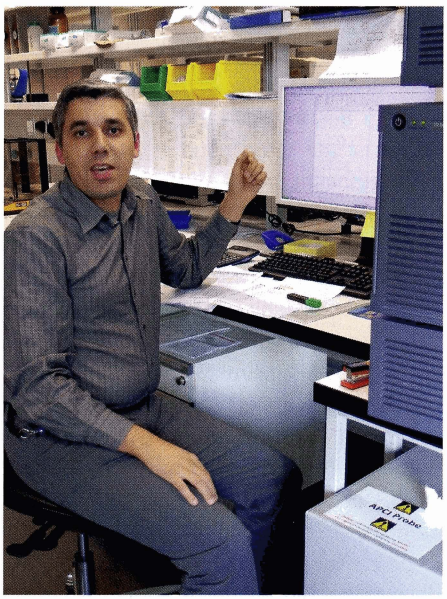
Seit dem Zweiten Weltkrieg haben natürliche oder von Naturstoffen abgeleitete und inspirierte Wirkstoffe massgeblich zum Erfolg der Pharmaindustrie beigetragen. Die damals lancierte grosstechnische Produktion von Penizillin führte zu grossangelegten F&E-Programmen, aus denen nicht nur Antibiotika wie Streptomycin, Gentamicin oder die Tetrazykline hervorgingen. Auch die Cholesterinspiegel senkenden Statine, die den Herstellern aktuell knapp 30 Milliarden Dollar Jahresumsatz bescheren, oder das cyclische Undecapeptid Ciclosporin, als Antibiotikum ein Flopp, als Immunsuppressivum in der Transplantationsmedizin ein Meilenstein, sind Produkte aus der Antibiotikaforschung.

Nicht nur aus mikrobiellen, auch aus pflanzlichen und anderen Extrakten wurden wertvolle Substanzen isoliert. Ein bekanntes Beispiel ist Paclitaxel, der Wirkstoff des weltweit umsatzstärksten Zytostatikums «Taxol». Das Molekül wurde Ende der 60er-Jahre beim US-amerikanischen National Cancer Institute in Extrakten der pazifischen Eibe gefunden und wird inzwischen teilsynthetisch ab nachwachsenden Nadeln und Zweigen der europäischen Eibe sowie biotechnologisch hergestellt. Von Naturstoffen abgeleitete Wirkstoffe machen auch in der Gegenwart von sich reden, u. a. vermeldete Novartis 2010 die Erstzulassung von «Gilenya». Der darin enthaltene oral verfügbare Wirkstoff Fingolimod ist ein synthetischer Abkömmling des Pilz-Stoffwechselprodukts Myriocin und war ursprünglich als Immunsuppressivum vorgesehen, nun verbessert er die Behandlung von Patienten mit schubförmig verlaufender Multipler Sklerose.

Nach einer drastischen Drosselung der industriellen Naturstoffforschungsaktivitäten in den 1990ern und frühen 2000ern hoffen Experten nun auf eine Kehrtwende und verweisen u. a. auf die Krebstherapie, wo gerade verschiedene neue Wirkstoffe aus der Naturstoffforschung zugelassen wurden bzw. entwickelt werden.



Basler Naturstoffforscher: Prof. Matthias Hamburger und Doktorandin Daniela Eigenmann.



Wissenschaftlicher Mitarbeiter Dr. Mouhssin Oufir am Massenspektrometer.

(Bilder: B. Peiseler-Sutter)

Naturstoffforschung als technologiebestimmter, schneller Prozess

«Mit dem Aufkommen moderner Drug Discovery Verfahren, der Forcierung des automatisierten Hochdurchsatz-Screenings und des stark beschleunigten Testens riesiger Substanzbibliotheken, die z. B. mittels kombinatorischer Chemie erzeugt werden, geriet die klassische Naturstoffforschung in den 90ern unter grossen Druck», erinnert sich Matthias Hamburger, der damals in den Aufbau des von Glaxo-Wellcome und dem Economic Development Board of Singapore getragenen, 2002 privatisierten Center for Natural Products Research (nun Merlion Pharma) in Singapur involviert war. Nach einer Professur in Jena ist Hamburger seit 2004 Professor für Pharmazeutische Biologie am Pharmazentrum der Universität Basel. Er hat massgeblich dazu beigetragen, dass die Naturstoffforschung inzwischen ein hochgradig technologiebestimmter und schneller Prozess ist.

Zwar bleibt das Aufspüren von Bioaktivität in komplexen mikrobiellen oder pflanzlichen Rohextrakten, die Hunderte, darunter viele strukturverwandte Naturstoffe in unterschiedlichsten Konzentrationen enthalten können, eine arbeitsintensive Herausforderung ohne Erfolgsgarantie. Die Miniaturisierung der Biotests, die Hochdurchsatz kompatible Formatierung von Extrakt- und Naturstoffsammlungen, die Parallelisierung des Screeningprozesses, bei dem die Fraktionierung der Proben, die Ausführung der Tests und die Analytik wo immer möglich nebeneinander laufen, bis hin zur stetigen Weiterentwicklung der chromatografischen und analytischen Methoden (Hochleistungsflüssigkeitschromatografie, Gaschromatografie, Massenspektrometrie, Kernresonanzspektroskopie…), haben die Naturstoffsuche in den letzten zwanzig Jahren aber deutlich effizienter gemacht.

Eine leistungsfähige und flexibel einsetzbare Technologieplattform im Rücken, spürt Hamburgers 20-köpfiges Team der Bioaktivität in meist pflanzlichen Extrakten nach und

verwendet dabei fast ausschliesslich Ganzzelltests. «Neue Ansätze findet nur, wer das Ziel nicht von Vornherein, z. B. durch die Verwendung biochemischer Tests, exakt definiert, weshalb wir auf sogenannte High Content Assays schwören. Hier wirken nur membrangängige Substanzen, eine wichtige Voraussetzung für oral verabreichbare Wirkstoffe. Auch zelltoxische Verbindungen outen sich dabei sofort», bekräftigt der Pharmazeut und unterstreicht, dass mikrobielle und pflanzliche Sekundärmetabolite unabhängig von ihrer Rolle als potenzielle Pharmaka wichtige Werkzeuge für die Grundlagenforschung sind, wo sie z. B. zur Modifizierung zellulärer Prozesse eingesetzt werden.

«Wir verstehen noch gar nicht, warum es diese Inhaltsstoffe überhaupt gibt, die den Produzenten höchstwahrscheinlich zur Kommunikation und Abwehr dienen. Auch durch die Aufklärung der enzymatisch katalysierten, stereoselektiven Vielschrittsynthesen, über die diese komplizierten asymmetrischen Moleküle biosynthetisiert werden, können wir viel lernen. Dass die Substrate dabei hoch selektiv an dreidimensional passenden, aktiven Enzymzentren umgesetzt werden, ist wohl der Grund dafür, dass sie oft auch mit strukturverwandten Biomolekülen artfremder Organismen wechselwirken und damit als Wirkstoffe solchen Erfolg haben», spekuliert der Wissenschaftler.

Umfangreiche Substanzsammlung

Seit zehn Jahren baut Hamburger eine eigene Substanzsammlung auf, die mittlerweile über 2000 Einträge zählt, hauptsächlich pflanzliche Extrakte, aber auch Einzelsubstanzen. Das Screening der Sammlung läuft in Kooperation mit Partnern, die entwicklungswürdige Zielstellen (emerging targets) in Ganzzell-Biotests darstellen und frühe Tests am Tiermodell ermöglichen. Lokal kooperieren die Basler Naturstoffforscher z. B. mit dem Schweizerischen Tropen- und Public Health-Institut (Swiss TPH), wo die Forschungsgruppe Parasite Chemotherapy mit ihrer Expertise auf dem Gebiet tropischer Krankheiten und den zu Grundlagenforschungszwecken entwickelten Kultur- und Testverfahren für Protozoen ein gefragter Projektpartner ist (vgl. Chemie plus 7/8-2011).

Hamburgers Gruppe hat bereits diverse bioaktive Naturstoffe entdeckt, welche als Forschungschemikalien im Handel sind, darunter ein Indolinon-Alkaloid, welches die Mastzelldegranulation hemmt, oder Indirubin, ein Kinase-Hemmstoff. Die Forscher adaptieren ihr Screening-Konzept an den jeweiligen Test, was viel Entwicklungs- und Validierungsarbeit bedeutet. Aktuell freuen sie sich über grosses Interesse an dem Leukotrien-Synthesehemmer Tryptanthrin, ein Alkaloid, welches ihnen vor zehn Jahren ins Netz ging und dessen entzündungshemmende Wirkung sich nun im Tiermodell bestätigt. Leukotriene sind Entzündungsmediatoren, die z. B. bei allergischen Erkrankungen eine zentrale Rolle spielen. Die Entwicklung von Inhibitoren, die das an der Leukotrien-Synthese beteiligte Schlüsselenzym 5-Lipooxygenase hemmen, war bisher wenig erfolgreich. Die einzige Ausnahme ist Zileuton («Zyflo» von Abott), ein 1997 in den USA zugelassener Wirkstoff mit stark lebertoxischen Nebenwirkungen.

«Tryptanthrin findet sich in Extrakten der ursprünglich asienstämmigen Färberwaid Isatis tinctoria, auch bekannt als deutscher Indigo. Nachdem wir bereits in zellulären Modellen zeigen konnten, dass das Molekül die Synthese von Leukotrien B4 hemmt, konnten wir die Wirkung nun nach oraler Gabe an Ratten mit induzierter Brustfellentzündung untermauern. Wir kennen den molekularen Angriffspunkt der Substanz noch nicht genau, der Wirkmechanismus ist aber auf jeden Fall anders als bei allen anderen bekannten Leukotrien-Synthesehemmern, was für die Grundlagenforschung interessant ist und der pharmazeutischen Forschung neue Anstösse liefern kann», resümiert der Entdecker.

Zusammen mit Forschern der Universität Wien ist sein Team Naturstoffen auf der Spur, die die Wirkung des GABA-A-Rezeptors modulieren. Der Rezeptor ist ein Chloridionenkanal auf Nervenzellen und wird vom Neurotransmitter Gamma-Aminobuttersäure (GABA) aktiviert, wodurch die zelluläre Aktivität sinkt. Auch Benzodiazepine, wie sie u.a. zur Behandlung von Angststörungen eingesetzt werden, docken an diesen Rezeptor an, allerdings an anderer Stelle, was die von GABA in Gang gesetzte Bremsung der Hirnaktivität weiter verstärkt. Weil Benzodiazepine diverse Nebenwirkungen zeigen und abhängig machen können, wird nach Alternativen gesucht. In einem gross angelegten Screening testeten die Basler Pharmazeuten die elektrophysiologischen Effekte von 880 Pflanzen- und Pilzextrakten auf GABA-A-Rezeptor-exprimierende Krallenfrosch-Eizellen. Dabei zeigte ein Extrakt des schwarzen Pfeffers (Piper nigrum) eine vielversprechende Wirkung. Der Scharfstoff Piperin wurde als aktive Substanz identifiziert, wobei über die HPLC-basierte Herangehensweise weitere aktive und inaktive Strukturanaloge identifiziert werden konnten. Daraus konnten wertvolle Informationen zur Struktur-Wirkungsbeziehung dieser Substanzklasse abgeleitet werden, die in ein medizinalchemisches Projekt eingespeist wurde. «Es ist uns damit gelungen, Piperin-Analoge mit höherer Aktivität und weniger Off-Target-Effekten zu finden», freut sich der Basler Forscher.

Oft ist es so, dass gleich mehrere Verbindungen in pflanzlichen Extrakten quasi im Team für eine wünschenswerte Wirkung verantwortlich sind, weshalb sich Hamburgers Gruppe nicht exklusiv der Leitstruktursuche widmet, sondern auch Phytopharmaka bis hin zu Nahrungsmitteln mit positiver Wirkung auf die Gesundheit unter die Lupe nimmt.

Interessantes Wildgemüse aus dem Wallis

Auf der Suche nach heimischen Pflanzen mit einem entsprechenden Mehrwert hat Doktorand Christian Abbet die alteingesessene Bevölkerung im Wallis zu ihren traditionellen Ernährungsgewohnheiten befragt und in diversen Archiven recherchiert. Dabei wurde er auf die Kugelige Teufelskralle bzw. Rapunzel Phyteuma orbiculare aufmerksam, die im Wallis traditionell als Wildgemüse Verwendung findet. Im Labor wurde ein ausführliches Profil der Inhaltsstoffe erstellt, darunter nicht nur wertvolle Carotenoide und Fettsäuren, sondern auch strukturell neuartige schaumbildende Saponine, die derzeit Gegenstand intensiver Untersuchungen sind. «Nicht nur in fernen Ländern, auch in Europa und der Schweiz geht traditionelles Wissen verloren», sorgt sich Matthias Hamburger. Die anwendungsorientierte Forschung, die den Walliser Bauern eine neue Einnahmequelle bescheren könnte, läuft in Zusammenarbeit mit Partnern wie den Agroscope-Forschungsanstalten des Schweizerischen Bundesamts für Landwirtschaft und soll nicht zuletzt auch zur Bewusstseinsbildung beitragen. ■

UCOHF
La Fouly

Le 4 mai 2011 s'est constituée à La Fouly l'Union des commerçants, partenaires touristiques et habitants de La Fouly et Val Ferret (UCOHF).

L'UCOHF est une association ouverte à laquelle peuvent adhérer toutes les personnes physiques ou morales exerçant ou non une activité lucrative, notamment les commerçants, artisans, cafetiers, restaurateurs, hôteliers, professions libérales, propriétaires de chalets, résidents.
L'UCOHF s'est donné pour buts d'organiser les animations de la station et participer, avec les organismes spécialisés, à des actions dans le domaine du tourisme, du sport ou de la culture;
de donner à la population et aux hôtes l'image d'un groupement actif et dynamique, soucieux d'une véritable qualité d'accueil;
de créer un climat de confiance et de concertation entre ses membres;
de défendre les intérêts de ses membres et ceux de leur clientèle.
Les membres du comité sont: Dominique Coppey Présidente, Marika Murisier Secrétaire-caissière, Alain Darbellay, Christophe Lonfat, Jean-François Thétaz, Candide Gabioud, Sibylle Bréaud, Marie-Bernard Bolis.
Vos suggestions, propositions, demandes d'adhésion et… vos coups de main seront les bienvenus. N'hésitez pas à nous contacter! à l'Office du Tourisme auprès de Marika Murisier au 027/783 27 17, qui nous transmettra vos suggestions.
Voici le programme des animations de cet été (renseignements complémentaires à l'office du Tourisme):

- 10.06 Inalpe en-dessous du Gîte de La Léchère possibilité de se restaurer au gîte
- 11.06 Inalpe Aux Ars – restauration sur place
- 02.07 Trail Verbier St-Bernard – Départ de La Fouly
- 09.07 Fête à Prayon au Restaurant le Dolent, chez les Bloyet
- 22.07 Concert à La Chapelle – Bella Musica – chansons interprétées par Stéphane Stas
- 23-24.07 Ben et sa petite Brocante
- 01.08 Grande Fête sous cantine, Gabidou le Clown, orchestre, fanfare, défilé, discours, feux d'artifice - sous réserve
- 6-7.08 Ben et sa petite Brocante
- 10.08 Concert à La Chapelle avec Anne et Florian Alter d'Orsières
- 12.08 Concert des Jeunes du Camp Musical en semaine de formation au Dolent
- 15.08 Fête de l'Assomption à Ferret, avec La messe en plein air et Concert – sous réserve –
- 26-27.08 Ultra Trail de Mont-Blanc
- 17.09 La Désalpe – date sous réserve, peut-être le 24.09
- 01.10 Fête de la Chasse à Prayon, au restaurant Le Dolent, chez Les Bloyet

Et tout l'été:
Sentier didactique sur les fleurs et les plantes.
Initiation au mur de grimpe, pour les enfants tous les mercredis.
animations hebdomadaires par le clown Gabidou avec ses ballons et certains jours ses ânes.
Animations musicales au Restaurant les Glaciers, chez Maurice et Mauricette, tous les dimanches.

Exposition photos
à La Fouly

Pour la troisième année consécutive, vous êtes invités à cheminer sur le sentier des marmottes agrémenté d'une exposition de photos. Après les papillons immortalisés par M Pichard, les photos anciennes du val Ferret tirées de la collection d'André Métroz, voici une troisième exposition consacrée aux plantes et fleurs autochtones. Doctorant à l'université de Bâle, Christian Abbet voue un amour particulier à notre flore alpine. Avec la collaboration active du prof. Hostettmann, son ancien professeur, il se fait un plaisir de vous présenter 13 «planches» évoquant les fleurs, leurs propriétés et leur utilisation éventuelle. Un autre regard sur nos plantes vous est proposé! En plus des traditionnelles photos, vous serez surpris d'apprendre les intuitions de nos ancêtres qui, bien avant les découvertes scientifiques, connaissaient les remarquables actions bienfaisantes ou nocives des végétaux sur l'organisme humain. Nous osons espérer que vous aurez du plaisir à les découvrir sous cet aspect à la fois visuel et documentaire.

Bonne promenade

Commission culturelle
M Abbet prés.

La Raiponce à l'honneur


Photo Université de Bâle

La raiponce, hôte toute discrète de notre région, fait ces temps-ci parler d'elle! Et ce, dans le cadre quelque peu huppé des laboratoires de l'Université de Bâle dirigés par le Professeur Hamburger. La raison? Elle vient de faire «une fleur» à l'un des habitants de notre commune, en lui «révélant» quelques-uns des secrets qu'elle renferme. Après avoir découvert deux molécules, Christian Abbet a eu l'honneur de publier le résultat de ses travaux dans une revue américaine de chimie des plus prestigieuses et le Poster Award 2011 de l'Annual Research Meeting à Bâle est venu couronner ce succès. Tour d'horizon avec le doctorant en biopharmacie.

La raiponce, qu'est-ce que c'est?

Une fleur alpine autrefois utilisée comme salade. Elle pousse abondamment dans notre région et embellit les pâturages d'alpages de sa parure bleue. Cette année, Walt Disney a d'ailleurs créé un dessin animé intitulé «Rapunzel» inspiré de l'œuvre des frères Grimm. Eh bien, il faut savoir que le scénario de ce conte repose certainement sur… la raiponce!

En quoi consiste votre travail?

A étudier des plantes comestibles de notre région tombées en désuétude. Cette activité se compose de deux volets: ethnobotanique et chimique. La première étape était de parcourir notre patrimoine à la recherche de végétaux intéressants. Des interviews de concitoyens, la lecture de livres anciens et de la littérature scientifique ont permis de sélectionner la raiponce parmi plus de 1100 plantes abordées. Je remercie au passage toutes les personnes que j'ai eu la chance de rencontrer et qui ont généreusement partagé avec moi leurs connaissances sur les plantes de notre région.

Une investigation chimique de la plante constitue la deuxième partie de la thèse. Cette étude aura pour but d'exclure les risques de toxicité ainsi que de mettre en évidence d'éventuels effets bénéfiques sur la santé.

L'étude des plantes alpines, un effet de mode?

Pas du tout. La tendance actuelle dans l'investigation de plantes est plutôt centrée sur les plantes exotiques et la médecine chinoise. Je suis un des seuls chercheurs à Bâle à travailler sur les plantes alpines mais reste persuadé du grand potentiel de celles-ci.

Que vous a apporté la raiponce?

La découverte de deux nouvelles molécules fort intéressantes qui, bien qu'indigènes sont chimiquement des plus exotiques!

Une découverte dans un univers scientifique qui en produit couramment?

Oui et non. Seul l'avenir nous le dira. D'un point de vue structurel, ces composés sont innovateurs.

Qui va pouvoir profiter de cette découverte, publiée maintenant à l'intention des chercheurs du monde entier?

Tout d'abord l'université et moi-même. Publier dans une telle revue reste des plus gratifiants. Mon travail sera également disponible sur internet. Peut-être trouvera-t-on aussi un jour des propriétés pharmacologiques intéressantes pour ces nouvelles substances. Enfin, notre région. Elle se voit nommée dans le travail ainsi que lors des congrès auxquels je participerai. Ce travail permet ainsi de valoriser notre patrimoine et de mieux le faire connaître.

En conclusion…

En tant que Valaisans, nous possédons une grande richesse dans notre flore unique et notre savoir ancestral. Cet héritage mérite d'être mis en valeur. C'est pourquoi, je vous invite à venir visiter cette année le parcours didactique de la Fouly qui sera justement consacré aux plantes indigènes.

L'avis de M. Hostettmann, ancien directeur du laboratoire de phytochimie à l'Université de Genève.

Des milliers de molécules d'origine végétale sont déjà connues. De ce fait en découvrir encore des nouvelles est un exploit en soi. Il faut aussi savoir qu'une plante peut contenir jusqu'à 10'000 substances chimiques différentes. Pour obtenir des substances pures et faire leur détermination de structure, c'est-à-dire leur identification, il faut maîtriser complètement des technologies sophistiquées afin d'établir la formule chimique. C'est un travail de longue haleine et très difficile qui demande de la patience, de la persévérance et de l'intelligence. Ces qualités, Christian Abbet les possède! Je l'ai eu comme étudiant en pharmacie à l'Université de Genève: il était brillant et a reçu un prix de la Société Suisse de Pharmacie en 2008 pour le meilleur travail de Master en pharmacie de l'année. Je ne suis donc pas très étonné que Christian continue dans la lancée pour obtenir des résultats excellents dans le cadre de sa thèse de doctorat à l'Université de Bâle. Les molécules qu'il a découvertes sont d'un type nouveau qui n'avait jamais été décrit jusqu'ici. Il s'agit donc d'une contribution importante à la connaissance de la chimie des plantes (phytochimie). De plus, ces molécules ont sans doute aussi un potentiel thérapeutique que Christian devra encore étudier en les soumettant à des batteries de tests biologiques et pharmacologiques. Je suis convaincu qu'on entendra encore parler des molécules découvertes par Christian. Enfin, alors que de nombreux chercheurs délaissent la flore indigène pour s'occuper de plantes exotiques qui ont le vent en poupe, le jeune chercheur d'Orsières a choisi d'étudier une plante bien de chez nous. Il a ainsi démontré que les plantes alpines ont encore bien des secrets à livrer. Si les nouvelles molécules de la raiponce devaient présenter des propriétés pharmacologiques importantes, on pourrait envisager de cultiver cette plante et contribuer ainsi à la diversification de notre agriculture de montagne.

4. Conclusions and outlook

In order to list edible plants consumed in canton of Valais, an ethnobotanical survey was performed in four different valleys of canton of Valais (Val d'Entremont, Val d'Illiez, Val d'Anniviers, and Val d'Hérens). Members of the older generation defined as age of 70 and above were specifically targeted, considering that the peoples' dependence on folk medicines and use of wild plants had declined considerably since 1940s. As a consequence of societal changes and urbanization the younger generation is indeed much less familiar with the traditional uses of wild plants. Historically, wild food plants were part of the diet and were of crucial importance in times of food scarcity. Meanwhile, they have lost their role as vital components of the diet, and are nowadays either perceived as delicacies.

98 food plants traditionally consumed in canton of Valais were mentioned by 38 informants and 27 further plants were extracted from reports and books written in the XIXth and XXth c. about alpine traditions.

Among the traditional food plants consumed in canton of Valais, two alpine plants namely *Phyteuma orbiculare* and *Cirsium spinosissimum* were selected for in depth analysis based on gustatory properties, their potential to be taken into cultivation and phytochemical profiling by HPLC-MS PDA analysis.

Phyteuma orbiculare flowers have been traditionally eaten by shepherds as sweeties, whereas leaf rosettes have been prepared as salads. The round-head rampion is a wide spread perennial plants and grows in alpine and subalpine meadows. As it is large enough, the plant may be a suitable candidate for future cultivation. No phytochemical data was available neither on the species nor on the entire genus.

Twenty-three compounds, including various phenolic glycosides, a new dimeric phenylpropanoid glucoside (tangshenoside VII), triterpenes and fatty acids were identified online, or after targeted isolation. In addition, two triterpene saponins, phyteumosides A and B, with structurally unique aglycons were isolated and characterized. The sapogenin of phyteumoside A can be considered as an incompletely cyclized onoceroid triterpene with two additional tetrahydropyran rings arising from oxygen bridges. The phyteumoside B possesses a new 17-polypodene aglycon. Interestingly, these saponins were only detected in flowers of *Phyteuma* spp. and were not detected in *P. hemisphaericum*. This demonstrates that alpine plants are still a promising source of structurally original secondary metabolites. No compounds with reported toxicity or substance classes with known toxicological risks were detected. Chromatographic profiles of *P.spicatum*, *P.ovatum* and *P.orbiculare* revealed

similar phytochemical compositions. This finding corroborates the parallel use of these three species as traditional food plants.

At the same time, our investigations revealed that *Phyteuma orbiculare* possesses interesting nutritive properties including favorable ω6:ω3 ratio, high contents in β-carotene and in minerals (potassium, calcium, and magnesium).

Based on their interesting chemical composition combined with pleasant gustatory properties, rampion species could be considered for future cultivation as food plants. Other *Phyteuma* species show bigger biomass (e.g. *P. spicatum*). However, this last specie grows in woods. Further agronomic investigations should be carried out to evaluate the breeding potential of *Phyteuma* species.

As second candidate, we selected the thistle *Cirsium spinosissimum*. The genus *Cirsium* is widely used as herbal medicine or food around the world. *C. japonicum,* for example, is used in traditional Chinese medicine (TCM) as an antihemorrhagic and diuretic agent. Young stems of *C. oleracea* are eaten as vegetable in Japan and India. In canton of Valais, *C. spinosissimum* was traditionally eaten by shepherds like artichokes. Surrounding leaves were removed to reach the edible part of the receptacle. *C. spinosissimum* grows abundantly in mountain regions, but no phytochemical information was available on the species. No phytochemical study was reported for this species.

Our study reveals the presence of various types of secondary metabolites including various phenolic compounds, fatty acids, a monoterpene lactone, and a spermine derivative. No compound with reported toxicity, nor substance classes with known toxicological risks were detected. The absence of cytotoxicity was confirmed by an *in vitro* assay on Caco-2 cell lines. In addition, the thistle may possess interesting nutritive properties according to its high amounts of potassium and magnesium. The plant contain also considerable amounts of two major flavonoids namely linarin and pectolinarin which have been reported to possess positive pharmacological effects.

Considering the chemical composition and pleasant gustatory properties of the receptacles, *C.spinosissimum* can be considered as a safe and nutritionally valuable food plant. The spiny leaves of the plant and its laborious preparation would, however, represent a real challenge for cultivation and harvesting.

In addition, this study allowed the identification of several further candidates which would be worth of a chemical investigation. These include the nutty bulb of *Bunium bulbocastanum*, the aromatic seeds of *Athamanta cretensis*, the bitter salad *Aposeris foetida*, and the spicy herb *Pritzelago alpina*.

The development of new crop has to meet several criteria and requires multidisciplinary collaboration. An important part of the work consists of the determination of phytochemical metabolite profiles. Such a work can corroborate the absence of toxicity of food plants, reveal the high content of substances relevant for nutrition, and finally help understanding possible pharmacological activities. *Phyteuma*, which combines an interesting chemical composition with pleasant gustatory properties, appears to be a promising candidate for future cultivation. For this purpose, seeds of the plant should be collected from different places of Valais to create heterosis and exploit all genetic features for the selection of varieties with interesting nutraceutical properties.

The establishment of alpine plants as food crops would represent a diversification of the activities in mountain agriculture. The Canton of Valais should profit of the actual trend for MAPs and continue to promote alpine plant development.

5. *Curriculum Vitae*

Curriculum vitae

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Formation

2009-2013	University of Basel, Basel, Switzerland **PhD Studies in the Division of Pharmaceutical Biology,** December 2013.
2006-2008	University of Geneva, Geneva, Switzerland **Master in Pharmacy,** October 2008.
2005-2006	University of Geneva, Geneva, Switzerland **Bachelor in Pharmaceutical Sciences,** September 2006.
2003-2005	University of Fribourg, Fribourg, Switzerland **Propaedeutics in Pharmacy,** July 2005.
1998-2003	College of the Royal Abbey of Saint-Maurice, Saint-Maurice, Switzerland **Matura in Latin-Sciences,** June 2003.

Professional experience

2011-2012	Setting up of a pharmacobotanical trail, Fouly (Switzerland).
2011	Poster Award 2011, Annual Internal Research Meeting, Université de Bâle (Suisse).
2010-2013	Military school as officer in GMP production.
2008-2012	Work as pharmacist in Pharmacie Frey, Romont.
2007	Master Thesis in Phytochemistry, University of Geneva.

Awards

2011	Poster Award 2011, Annual Internal Research Meeting, University of Basel.
2007	Best Master Thesis in Pharmacy, University of Geneva.

Personal skills

Languages	French	Mother tongue
	English	Level B2
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Informatics program	Windows Office 2010 (Word, Excel, PowerPoint), Photoshop, Illustrator CS Chemdraw Ultra, ACD Labs, Bruker, Hystar, Empower.	

Publications et Press

Publications

A AR Silva, MM Bezerra, HV Chaves, K MA Pereira, JA Aguiar, V PT Pinto, **C Abbet**, CA Simoes-Pires, ES Franco, AT Henriques, K Hostettmann, M BS Maia. (2012). J. Nat. Med., in press. *Protective effect of Chresta martii extract against indomethacin-induced gastric lesions in mice* .

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Presse

J Mosimann, *Besuch bei den Murmeltieren*, **Schweizer Illustrierte** (02.09.2012).

A Bochatay, *Elle m'a dit d'aller siffler là-haut*, **Le Nouvelliste** (07.07.2012).

S Imsand, *Les plantes qui soignent ont le vent en poupe*, **Le Matin** (28.06.2012).

B Peiseler-Sutter, *Hightechsuche nach Naturstoffen*, **Chemie Plus** (01.02.2012).

P Mayoraz, *Sous le signe du défi*, **Le Nouvelliste** (18.11.2011).

S Olff, *Flora Alpina*, **Sonntagsteitung** (18.09.2011).

O Schneider, *Un Valaisan distingué*, **Le Nouvelliste** (18.11.2007).

Television

S Olff, *Die Heilkraft der Natur, Schweizer suchen nach pflanzlichen Wirkstoffen*, **Nano** (3Sat) (28.08.2012).

S Olff, *Die Apotheke in den Alpen*, **Einstein** (SF1) (06.09.2012).

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