Chapter 2
Ways the Lukomir Highlanders of Bosnia and Herzegovina Treat Diabetes

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Dedicated to the memory of Sulejman Redžić.

2.1 Introduction

Today, diabetes is a pandemic disease and a top health concern in indigenous societies. The global burden of diabetes was estimated to affect 366 million people in the year 2011; by 2030, 552 million people are expected to have diabetes (IDF

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2013). Type 2 diabetes (T2D; formerly called noninsulin-dependent or adult-onset diabetes) results from the body's ineffective use of insulin and is characterized by hyperglycemia. T2D is responsible for 90 % of diabetes cases (Alberti and Zimmet 1998) and is linked to rapid social change, genetics, dietary acculturation, excess carbohydrate consumption, physical inactivity, and excess body weight (Young 1994; Hegele et al. 1999; Young et al. 1992, 2000; Ritenbaugh and Goodby 1989; WHO 2013a). The World Health Organization (WHO) estimated that Bosnia and Herzegovina (B&H) had 111,000 people with diabetes in 2000 and was expected to increase to 180,000 by 2030 (WHO 2013b).

Long-term complications of T2D include vascular disease, heart disease, stroke, neuropathy, retinopathy, cataracts, atherosclerosis, nephropathy, and impaired wound healing. Impaired glucose uptake affects the cells of organs that do not require insulin for glucose uptake (the nervous system, heart, kidneys, and small blood vessels). As a consequence, these cells have high concentrations of intracellular glucose during elevated hyperglycemic periods, resulting in impaired cell function and cell death (Ahmed 2005). These T2D complications are mediated by the formation of advanced glycation end products (AGEs), which are a therapeutic target with phytotherapies.

T2D incidence is three times higher in indigenous populations. These are some of the fastest-growing yet most vulnerable populations in the world that often lack culturally appropriate primary health care (Alberti et al. 2004; Ahmed 2005; Helin 2006). This is true for the Lukomir Highlanders of B&H, one of the last native communities in Europe, located in the Bjelašnica region of the Dinaric Alps (43.6°lat, 18.1°long, 1460 m.a.s.l.), southwest of the capital city Sarajevo. Local health authorities have described diabetes and heart disease as the most prevalent diseases in Lukomir (Ferrier et al. 2013). One reason for the prevalence of diabetes in Lukomir is due to a transition from traditional to higher-glycemic diets. Municipal water diversion was a driver for this transition in Lukomir. This recent postwar development project removed the source of water from Lukomir's cereal hydro mills, which in turn caused the collapse of the mills. This led to a nutrition shift from a traditional organic multigrain diet to a higher glycemic diet based on soft white wheat flour (Ferrier et al. 2013). This development has eroded the Highlanders' traditional lifestyle and exercise, transformed habitats of medical flora, and increased the prevalence of T2D.

Since Lukomir has no primary health-care facility, our objective was to identify the plants the Lukomir Highlanders use for the treatment of diabetes and highly associated symptoms. The plants were ranked using the syndromic importance value (SIV) function developed by Leduc et al. (2006) for a study of Cree traditional medicines. To assist future ethnobotanical studies, we present physician-ranked diabetes symptom weights (w) required for completing Leduc's SIV function. As a pilot pharmacology and phytochemistry study, we investigated the bioactivity and phytochemistry of one genus that was prominent in the Lukomir pharmacopoeia and cross-culturally used by our Eeyou Istchee Cree partners.

This ethnobotanical study of plants was conducted between 2007 and 2013 with the Lukomir community in Bosnia and Herzegovina, and J.F. and L.Š.'s organization, Cross-cultural Health Initiative (CHI). An international collaboration was developed with the Partnerships for Tomorrow Program, Phase II (PTP) funded by

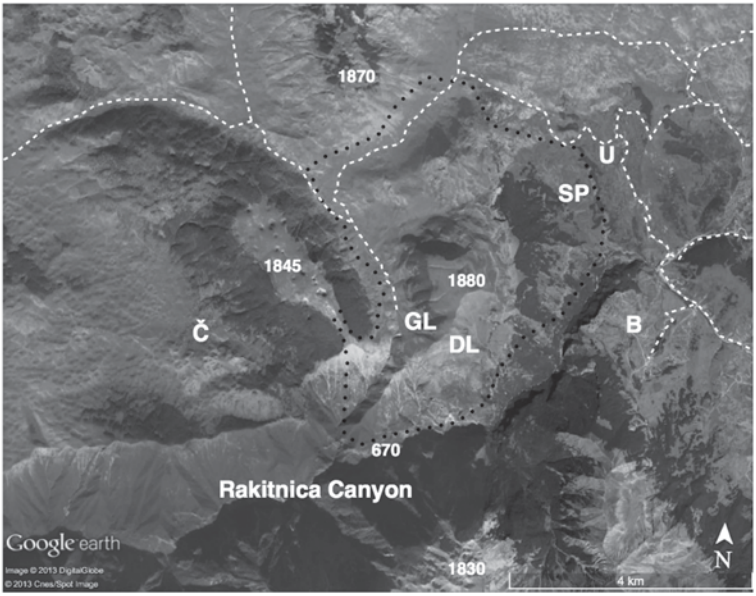

Fig. 2.1 Lukomir and nearby villages in the Bjelašnica region of Bosnia and Herzegovina. Taxa in Table 2.1 were collected within *black dotted perimeter* (~26 km^2). *White dashes* indicate roads leading to Lukomir. *Numerals* indicate meters above sea level (m.a.s.l.). Bobovica (*B*=1300 m.a.s.l.), Čuhovici (*Č*=1330 m.a.s.l.), Donji Lukomir (*DL*=1250 m.a.s.l.), Gornji Lukomir (*GL*=1460 m.a.s.l.), Studeni Potok (*SP*=1420 m.a.s.l.)

the Canadian International Development Agency (CIDA). Members of CHI were hosted by the University of Sarajevo and Foundation GEA+. Research permits were issued by the Municipality of Konjic and University of Ottawa (H05-09-07), with prior informed consent from Lukomir's leaders and informants.

2.2 Methods

2.2.1 Field Research

2.2.1.1 Partnership, Permits, and Prior Informed Consent

This ethnobotanical study of plants was conducted between 2007–2013 with the Lukomir community in Bosnia and Herzegovina, and J.F. and L.Š.'s organization, Cross-cultural Health Initiative (CHI). An international collaboration was developed with the Partnerships for Tomorrow Program, Phase II (PTP) funded by the Canadian International Development Agency (CIDA). Members of CHI were hosted by the University of Sarajevo and Foundation GEA+. Research permits were issued by the Municipality of Konjic and University of Ottawa (H05-09-07), with prior informed consent from Lukomir's leaders and informants.

2.2.1.2 Study Site: Lukomir, Municipality of Konjic, Bosnia and Herzegovina

A consensus ethnobotany was conducted in the Bjelašnica area of the Dinaric Alps, in Lukomir, with the Lukomir Highlanders of Bosnia and Herzegovina (Fig. 2.1). This area is classified as an alpine biogeographic region that is closely bordered by Mediterranean and continental biogeographic regions (European Environment Agency (EEA) 2012). Many community members are descendants of a Bogomil lineage who first settled in *Donji Lukomir* (Lower Lukomir; 43.632 lat, 18.194 long, 1200 m.a.s.l.) and eventually moved to *Gornji Lukomir* (Upper Lukomir, commonly referred to as Lukomir; 43.637 lat, 18.182 long, 1460 m.a.s.l.). Lukomir's informants included spiritual leaders, elders, younger women, and men. Informants described plants on field trips, garden tours, while shepherding, or in comfortable settings of their choice. We earned our interview time with Lukomir's healers by volunteering our time to shepherd, harvest food, and stack hay. This allowed for participatory observation, and gave informants more time on field collection trips and interviews. Notes and photos were taken when participants displayed preparation methods of plant and natural product remedies.

Land mines were avoided by consulting with Bosnia and Herzegovina Mine Action Center (BHMAC; http://www.bhmac.org). All plants were collected on trail sides or in areas that were constantly traveled by sheep herds, since only parts of the Lukomir territory were surveyed by BHMAC.

Field work followed a quantitative consensus methodology with individual semistructured interviews during which L. Šačiragić, J. Ferrier, and S. Redžić collected the following data: (1) specimen voucher number, (2) photo number, (3) common name, (4) scientific name, (5) family, (6) GPS coordinates, (7) altitude (m.a.s.l.), (8) habitat, (9) syntaxa, (10) flowering time and description, (11) medically active collection time, (12) use, (13) use category, (14) plant part used, (15) amount used, (16) preparation method, (17) administration method, (18) dosing regimen, (19) ethnographic details, (20) informant name, and (21) date. Determinations were made using a Domac's regional flora (1984), vouchers, and Tropicos.org (2013). Duplicate vouchers were collected (when sustainable) and are currently held at the University of Ottawa Herbarium (OTT) with voucher numbers reported in Table 2.1. There are plans for a herbarium in Lukomir to assist future botanical studies. Vouchers of this study will be placed in the Lukomir herbarium when available. Plant vouchers and an iPad (Apple, Cupertino, USA) were used to display collections to elders who could not venture over the mountainside, or for informant review purposes.

2.2.1.3 Elucidation of Plants for Diabetes Using Syndromic Importance Values

The SIV function was adapted from Leduc et al. (2006), Oubré et al. (1997), and McCune and Johns (2002, 2003). SIVs allow ranking of plant species by accounting for (1) the number of different symptoms for which a plant was cited, (2) the frequency of plant citation by individual informants, and (3) the association rank

Table 2.1 Taxa used by the Lukomir Highlanders to treat symptoms of diabetes. Dagger (†) indicates endemism. Determinations followed legitimate names in Tropicos.org (2013)

Scientific name	†	Common name	Symptom	V#	SIV
Achillea millefolium L.	–	Kunica	Diabetes	358	0.00725
Achillea millefolium L.	–	Kunica	Swelling or inflammation	358	0.00725
Anthyllis vulneraria L.	–	Ranjenik	Slow healing infections	372	0.00095
Asarum europaeum L.	–	Kopitnik, kopitnjak	Slow healing infections	382	0.00089
Capsella bursa-pastoris (L.) Medik.	–	Rustemača	Swelling or inflammation	398	0.00289
Cetraria islandica (L.) Ach	–	Islandski lišaj	Heart or chest pain	403	0.00192
Cichorium intybus L.	–	Konjanik	General weakness	411	0.00235
Cornus mas L.	–	Drijen	Slow healing infections	384	0.00089
Crataegus monogyna Jacq.	–	Glog, gloginje	Back or kidney pain	361	0.00553
Crataegus monogyna Jacq.	–	Glog, gloginje	Diarrhea	361	0.00553
Elymus repens (L.) Gould	–	Pirika	Heart or chest pain	389	0.00299
Elymus repens (L.) Gould	–	Pirika	Increased urination	389	0.00299
Equisetum arvense L.	–	Preslica	Back or kidney pain	367	0.00588
Equisetum arvense L.	–	Preslica	Swelling or inflammation	367	0.00588
Gentiana lutea L.	†	Lincura	Sore or swollen limbs	393	0.00257
Jovibarba hirta (L.) Opiz	†	Čuvarkuća	Slow healing infections	379	0.00363
Jovibarba hirta (L.) Opiz	†	Čuvarkuća	Swelling or inflammation	379	0.00363
Matricaria matricarioides (Less.) Porter ex Britton	–	Kamilica, Stomaklija	Diabetes	351	0.00775
Matricaria matricarioides (Less.) Porter ex Britton	–	Kamilica, Stomaklija	Swelling or inflammation	351	0.00775
Mentha longifolia (L.) L.	–	Nana	Swelling or inflammation	349	0.00428
Nepeta cataria L.	–	Macina trava	Swelling or inflammation	369	0.00321
Ononis spinosa L.	–	Gladišika	Increased urination	377	0.00115
Phyllitis scolopendrium (L.) Newman	–	Podrebnica (♂ or ♀)	Heart or chest pain	357	0.00259
Plantago lanceolata L.	–	Bokvica ♀	Heart or chest pain	359	0.00304
Plantago lanceolata L.	–	Bokvica ♀	Slow healing infections	359	0.00304
Plantago major L.	–	Bokvica ♂	Heart or chest pain	360	0.00304
Plantago major L.	–	Bokvica ♂	Slow healing infections	360	0.00304
Polygonum bistorta L.	–	Srčanik	Heart or chest pain	356	0.00266
Primula veris L.	–	Jagorčevina	Diabetes	373	0.00382
Prunus spinosa L.	–	Trnjina	Blurred vision	405	0.00428
Prunus spinosa L.	–	Rakija	Swelling or inflammation	405	0.00428
Rubus saxatilis L.	–	Kupina	Diabetes	407	0.00342
Salvia officinalis L.	†	Kadulja	Heart or chest pain	348	0.00296
Sambucus wightiana Wall. ex Wight & Arn.	–	Haptovina	Heart or chest pain	376	0.00192
Sambucus nigra L.	–	Zova, zobovina	Heart or chest pain	354	0.00266
Satureja montana L.	–	Vrijesak	Diabetes	366	0.00408
Sedum sexangulare L.	–	Zednjak	Slow healing infections	416	0.00086

Table 2.1 (continued)

Scientific name	†	Common name	Symptom	V#	SIV
Silene uniflora Roth ssp. *glareosa* (Jord.) Chater & Walters	†	Puca	Heart or chest pain	353	0.00741
Silene uniflora Roth ssp. *glareosa* (Jord.) Chater & Walters	†	Puca	Increased urination	353	0.00741
Silene uniflora Roth ssp. *glareosa* (Jord.) Chater & Walters	†	Puca	Slow healing infections	353	0.00741
Silene uniflora Roth ssp. *glareosa* (Jord.) Chater & Walters	†	Puca	Swelling or inflammation	353	0.00741
Silene uniflora Roth ssp. *prostrata* (Gaudin) Chater & Walters	†	Puca	Heart or chest pain	350	0.00741
Silene uniflora Roth ssp. *prostrata* (Gaudin) Chater & Walters	†	Puca	Increased urination	350	0.00741
Silene uniflora Roth ssp. *prostrata* (Gaudin) Chater & Walters	†	Puca	Slow healing infections	350	0.00741
Silene uniflora Roth ssp. *prostrata* (Gaudin) Chater & Walters	†	Puca	Swelling or inflammation	350	0.00741
Smyrnium perfoliatum L.	–	Ljaljica	Increased urination	380	0.00111
Solanum tuberosum L.	–	Krompir	Swelling or inflammation	408	0.00278
Symphytum officinale L.	–	Gavez	Slow healing infections	383	0.00089
Teucrium montanum L.	–	Iva	Diabetes	352	0.00487
Tilia platyphyllos Scop.	–	Lipa	Heart or chest pain	374	0.00215
Tussilago farfara L.	–	Podbijel (♂ or ♀)	Heart or chest pain	371	0.00215
Urtica dioica L.	–	Žara, kopriva	Heart or chest pain	362	0.00222
Urtica dioica L.	–	Žara, kopriva	Slow healing infections	362	0.00222
Vaccinium myrtillus L.	–	Borovnica	Diabetes	368	0.00480
Vaccinium vitis-idaea L.	–	Brusnica	Diabetes	385	0.00441
Vaccinium vitis-idaea L.	–	Brusnica	Slow healing infections	385	0.00441
Verbascum thapsus L.	–	Divizbina, divizma	Heart or chest pain	390	0.00200
Vitis vinifera L.	–	Sirce	Swelling or inflammation	409	0.00278

of symptoms for which a plant was cited to treat. Four physicians at The Ottawa Hospital, Ottawa, Canada, who diagnose and treat patients with diabetes, determined the latter association rank of symptoms. Symptoms were given a weight (w) from 1 to 4, where 1 is a symptom highly associated with diabetes; 2, moderately associated with diabetes; 3, weakly associated with diabetes; and 4 is not at all associated with diabetes:

Table 2.2 *Vaccinium* spp. and outgroup *A. uva-ursi* leaf samples collected in Jahorina, B&H, with IC_{50} expressed in $\mu g \cdot mL^{-1}$. All sample vouchers were accessioned at University of Ottawa, OTT

Sample	IC_{50}	Location	Alt. (m)	Date	Voucher	Extract #
V. myrtillus	4.1	Igman, B&H	984	July 1, 2005	417	BBE 134
V. myrtillus	5.4	Jahorina, B&H	1730	July 24, 2005	418	BBE 42
V. myrtillus	17.35	Jahorina, B&H	1730	July 1, 2005	419	BBE 133
V. myrtillus	27.8	Jahorina, B&H	1730	July 5, 2005	420	BBE 125
V. vitis-idaea	48.6	Jahorina, B&H	1730	June 5, 2006	421	BBE 23
A. uva-ursi	100.2	Jahorina, B&H	1735	June 15, 2006	422	ARC 1

$$\text{SIV} = \frac{\left[\frac{\sum ws}{S}\right] + \left[\frac{\sum wf}{SF}\right]}{2} = \frac{\sum ws + \left[\frac{\sum wf}{F}\right]}{2S}.$$

The SIV function accounts for *w*, the weight of the symptom; *s*, the symptom treated by the species; *f*, the frequency of citation for the species; *S*, the total number of symptoms used for the survey (used in Leduc et al. 2006, but was not indicated in the denominator on the bottom right); and *F*, the total number of interviews in the survey.

2.2.1.4 Statistics and Phylogenetic Analysis

Statistical analysis was conducted using Prism 6 software. A phylogenetic tree was constructed using TreeGraph 2.0.47-206 beta, taxon with positive SIVs, and based on circumscription and topology presented by Angiosperm Phylogeny Group 3 (APG 3 2009) and the Linear Angiosperm Phylogeny Group 3 (LAPG 3; Haston et al. 2009).

2.2.1.5 Cross-Cultural Consensus

In collaboration with the Eeyou Istchee Cree and their Canadian Institute of Health Research Team for Aboriginal Antidiabetic Medicines (CIHR TAAM), we compared the Lukomir ethnobotanical taxa with the Cree CIHR TAAM's taxa (Leduc et al. 2006) to find consensus specimens for cross-culturally relevant pharmacological and phytochemical investigations. Both communities contain alpine and continental-type habitats and are isolated linguistically and geographically from each other.

2.2.2 Laboratory Research

2.2.2.1 Extraction of Consensus Specimen and Outgroup Leaf Material

Vaccinium spp. and outgroup *Arctostaphylos uva-ursi* (L.) Spreng. leaves were collected and identified by S. Trakić, S. Redzić, and J. Ferrier, and deposited at the OTT (Table 2.2). Samples were dried at 35 °C and ground in a Thomas Wiley mill (1 mm mesh). Plant material was extracted twice in ethanol/water (95:5 %) at room temperature for 24 h per phase. The phases were centrifuged at 1000 ×*g*, filtered, pooled, dried using a Speed Vac (Savant, Halbrook, NY), and lyophilized (Edwards Super Modulyo Freeze Drier, Fisher Scientific, Ottawa, Canada).

2.2.2.2 Inhibition of Advanced Glycation End Products by Consensus Genera Species

Inhibition of AGE formation was assessed as described by Farsi et al. (2008) with modifications. Bovine serum albumin (BSA; 1 mg mL^{-1}) was incubated with 100 mmol L^{-1} glucose/ 100 mmol L^{-1} fructose in sodium phosphate monobasic monohydrate buffer (pH 7.4) with extract (experimental treatment), ethanol/water (4:1) (negative control), or quercetin, an antioxidant against glycation by way of phenolic hydroxyl groups in the flavonoid structure (24 µg mL^{-1} in assay; Sengupta et al. 2006), which served as a positive control. To control for fluorescence of extracts, a treatment without BSA was included. To control for fluorescence of BSA, a treatment with BSA and vehicle was included. Stock solutions of extracts were serially diluted and tested at five concentrations that were optimized for dissolution and a linear concentration response (40, 20, 10, 5, and 2.5 µg mL^{-1} in well concentration). Three replicates were tested in sterile opaque polystyrene 96-well clear bottom plates (Corning Inc., New York, NY, USA). Plates were covered, sealed with Parafilm, and incubated for 7 days at 37 °C while shaking. Following incubation, fluorescence was measured using a microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, CA, USA) at excitation and emission wavelengths of 355 and 460 nm. Glucose/fructose and ethanol/water fluorescence was subtracted from all results, and percent inhibition and IC_{50} values were calculated as previously described (Farsi et al. 2008).

2.2.2.3 HPLC–MS Analysis of Vaccinium Species

Stock solutions of extracts were prepared to 10 mg mL^{-1} in ethanol/water (80 %:20 %) and filtered through a 0.2 µm PTFE nonsterile filter (Chromatographic Specialties Inc.). High-performance liquid chromatography (HPLC) analyses were performed using an Agilent 1100 chromatographic system (Agilent Technologies Inc.) consisting of an autosampler, quarternary pump, and diode array detector (DAD). Solvents were of HPLC grade (Fisher Scientific), and trifluoroacetic acid (TFA) was of analytical grade (J.T. Baker). A Syngeri RP-Polar column (150 mm × 3 mm; 4 μm particle size) was kept at 53 °C and a flow rate of 0.5 mL min^{-1} was maintained. The mobile phase consisted of aqueous TFA (0.05 %; solvent A), acetonitrile with TFA (0.05 %; solvent B), and methanol (solvent C). Initial conditions were set at 95:5:5 % (A:B:C) and changed following a linear gradient of 5–9.2 % B and 5–17.5 % C in 25 min. The column was then washed by increasing solvent B to 100 % over 5 min and returned to initial conditions in the next 5 min. The column was allowed to re-equilibrate for 5 min, resulting in a total run time of 40 min. Ten microliters of each 10 mg mL^{-1} leaf extract were injected for each run, and the elution profiles were monitored at 350 , 280 , and 230 nm with bandwidth kept at 4.

Phytochemical constituents were identified based on comparison to retention times and ultraviolet (UV) spectra of pure standards (95 % purity) relative to a programmed library of known UV spectra, and further confirmed by mass spectrometry (MS) fragmentation patterns. Standard compounds used to monitor leaf extracts were (+)-catechin, chlorogenic acid, quercetin-3-O-glucoside, (Extra-synthèse), rutin, quercetin-3-O-rhamnoside (Sigma) paracoumeric acid, taxifolin, quercetin-3-O-galactoside, and myricetin (source unknown).

2.3 Results and Discussion

We were able to interview 25 informants who described plant uses on mountain and canyon field trips by volunteering to shepherd, collect food, and hay with the Highlanders between 2008 and 2010. Informants provided information on diabetic symptoms and did not initially provide any diabetes use reports, but during subsequent conversations on casual visits to Lukomir, many healers agreed that panacea remedies should be used to treat diabetes. Highlanders described eight panacea treatments, which were subsequently treated as a diabetes SIV use report.

All eight species used as "diabetes" treatments were cited as *čaj* (infusions). In one household's case, we noticed that some of these taxa were present in their *čaj* collection, but were not cited as medicine: *Teucrium montanum* L., *Salvia officinalis* L., and one specimen never mentioned, *Phyllitis scolopendrium* (L.) Newman, (*podrebnica*[1]). When we noticed this *čaj* collection, we asked why they were not mentioned. The response: "We drink *čaj* for our health." These plants were for their "medicine pot," that were prepared with a prayer after meal consumption. This household also has one family member with T2D managed by prescription medicines. Perhaps these plants are best referred to as Pieroni and Quave (2006) describe

[1] Redžić (2008) translated Podrebnica, "under the ribs," and explained that the name refers to the fern's sori aligned in a chevron-like pattern on the ventral side of the frond, which resembles a rib cage. This pattern spurred the doctrine of signatures for usage of *P. scolopendrium* as a treatment for ailments under the ribs.

them: “medicinal foods or food medicines”—prepared to obtain “medical action,” consumed in a “food context,” and in this case, not always cited as medicine.

The Lukomir Highlanders used 41 species for the treatment of diabetes symptoms (see Table 2.1). Antidiabetic reports (in parentheses) were of plants situated in five general habitats: grassland (21), village and shepherd trails (12), mountain slopes (9), riparian zones (6), rocklands (6), deciduous forest (4), and cultivated (2). Antidiabetic preparation methods were infusion (46), poultice (7), food (3), beverage (2), ear drops (2), juice (1), foot bath (1), eye wash (1), and tincture (1). The frequency of taxa to treat diabetes and associated symptoms were heart and chest pain (15), swelling or inflammation (13), slow healing infections (12), diabetes/panacea (8), increased urination (5), back or kidney pain (2), diarrhea (1), blurred vision (1), general weakness (1), and sore or swollen limbs (1).
The Lukomir Highlanders mentioned five endemic species (†, Fig. 2.2). Endemic taxa account for 20% of use reports: *Jovibarba hirta* (L.) Opiz (Crassulaceae), *Silene uniflora* ssp. *glareosa* (Jord.) Chater & Walters, *Silene uniflora* Roth ssp. *prostrata* (Gaudin) Chater & Walters (Caryophyllaceae), *Salvia officinalis* L. (Lamiaceae), and *Gentiana lutea* L. (Gentianaceae) were also listed as endangered (see Fig. 2.2).

2.3.1 Ways the Lukomir Highlanders Treat Diabetes and Associated Symptoms

In order to rank taxa from interviews with the SIV function, the weight (*w*) of diabetes and 15 associated symptoms were given association to diabetes values by four physicians: increased thirst (1), slow healing infections (1), increased urination (1.25), foot numbness or foot sores (1.25), blurred vision (1.75), diarrhea (2.25), heart or chest pain (2.25), abscesses or boils (2.25), frequent headaches (2.75), general weakness (2.75); increased appetite (2.75) and sore or swollen limbs (3); arthritis or rheumatism (3.25) and back or kidney pain (3.25); and swelling and/or inflammation (3.25) and diabetes (4).

SIVs of species were presented in Table 2.1. The top five SIV factors (in parentheses) belonged to *Matricaria matricarioides* (Less.) Porter ex Britton (0.0078), *Silene uniflora* ssp. *glareosa* (0.0074), *Silene uniflora* ssp. *prostrata* (0.0074), *Achillea millefolium* L. (0.0073), and *Equisetum arvense* L. (0.0059). Each species’ SIV was multiplied by 100,000 and presented as wedges to infer phylogenetic importance at various taxonomic levels (see Fig. 2.2). The top five families with the highest average SIVs were Caryophyllaceae (0.0074), Equisetaceae (0.0059), Asteraceae (0.0049), Ericaceae (0.0046), and Rosaceae (0.0044). Considering the factors and framework of the SIV function, families and genera in Fig. 2.2 with large SIV wedges have potential in delivering new medicines for diabetes. Furthermore, studies have indicated that targeting closely related taxa is a sufficient strategy for bioactivity screening (Cox and Balick 1994; Rønsted et al. 2008; Saslis-Lagoudakis et al. 2011). However, taxa that have strong phylogenetic signal (see Fig. 2.2) plus cross-cultural consensus have been demonstrated to contain more bioactive plants than random samples (Saslis-Lagoudakis et al. 2012).

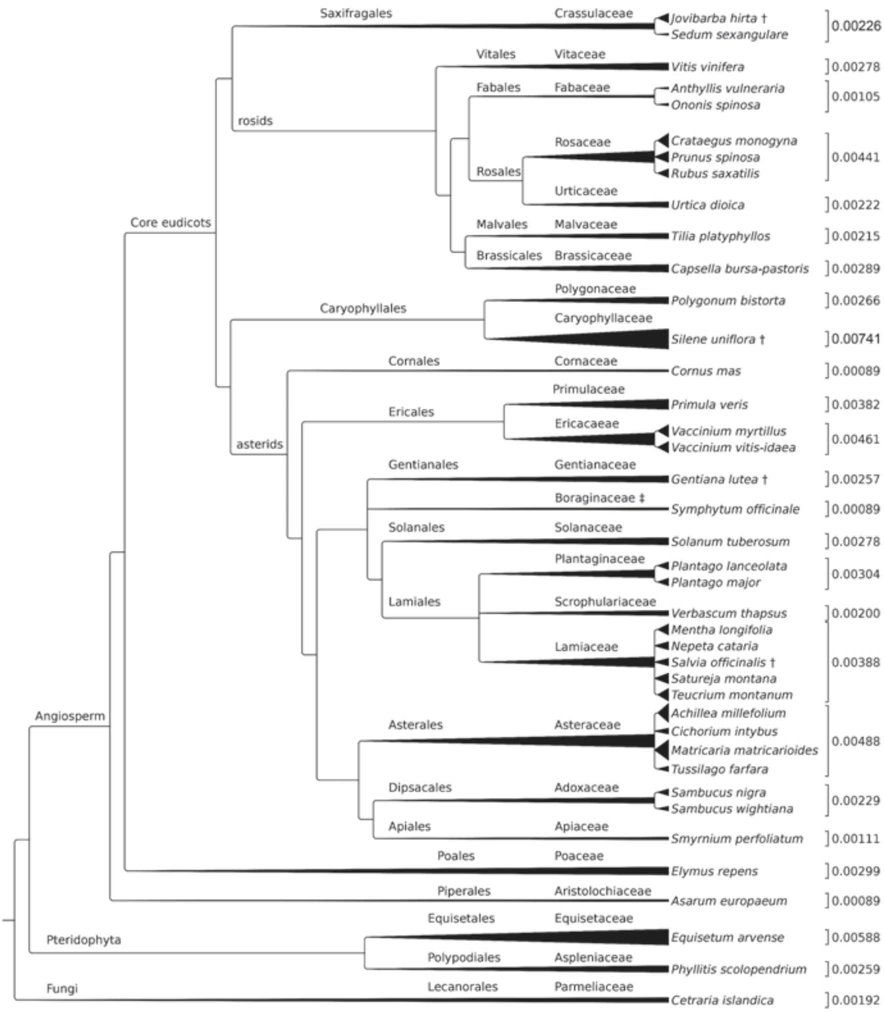

Fig. 2.2 Clustering of taxa used to treat diabetes and associated symptoms of diabetes. Average SIVs for each family are presented and *daggers* (†) indicate endemic taxa. *Double daggers* (‡) represent orders not delimited by the APG 3 (APG 3 2009). Topology is based on LAPG 3 (Haston et al. 2009). Branch lengths are not to scale

2.3.2 An Intriguing Cross-Cultural Consensus Among the Lukomir Highlanders and the Cree of Eeyou Istchee

Two consensus families emerged when Leduc's study (2006) was compared with Table 2.1: Ericaceae and Rosaceae. *Vaccinium* of the Ericaceae was the only genus with cross-cultural consensus. There was one consensus species, *V. vitis-idaea* L., which was used by three Cree healers and two Lukomir healers for slow-healing infections and urinary infections. *Vaccinium myrtillus* L. was used by four Highlander healers as a panacea. *V. angustifolium* Ait. was used by three Cree healers for frequent headaches. SIV factors from Lukomir ranked *V. myrtillus as* 8/41 plants and *V. vitis-idaea as* 9/41 plants (see Table 2.1). SIV factors from the Cree survey ranked *V. angustifolium* as 11/15, and *V. vitis-idaea* as 13/15. SIVs indicate that *Vaccinium* spp. are more important for the Highlanders than the Cree for treating diabetes.

2.3.3 Inhibition of AGEs

Both *V. myrtillus* and *V. vitis-idaea* showed linear concentration-dependent inhibition of AGEs with activity higher or comparable to the positive control quercetin (see Table 2.2). *V. myrtillus* ($n=3$ at 50 µg mL^{-1} initial concentration and $n=1$ at 40 µg mL^{-1} concentration) had an average IC_{50} of 12.4 µg $mL^{-1,}$ and *V. vitis-idaea* ($n=1$) had an IC_{50} of 48.6 µg mL^{-1}. The data for the most active species, *V. myrtillus*, are shown (Fig. 2.3). *Vaccinium* spp. herein had approximately 61 % greater activity than outgroup *A. uva-ursi* ($IC_{50=}$100.2 µg mL^{-1}). These results were comparable to $IC_{50\text{'s}}$ from wild samples of *V. angustifolium* Aiton (McIntyre et al. 2009) ranging from 4.8 to 10.6 µg mL^{-1} with a seasonal mean of 6.3 µg mL^{-1}. *Vaccinium poasanum* Donn. Sm. was the most active taxon ($IC_{50=}$4.2 µg $mL^{-1)}$ in an environmental and date-controlled study consisting of numerous tropical specimens (Ferrier et al. 2012). Considering this comparison, and knowing that AGEs contribute to the development of retinopathy, cataracts, atherosclerosis, neuropathy, nephropathy, diabetic embryopathy, and impaired wound healing, the Highlanders' access to and use of *V. myrtillus* (Borovnica) is appropriate as a panacea remedy and may be an effective means of preventing AGE-related insults, which should be studied clinically.

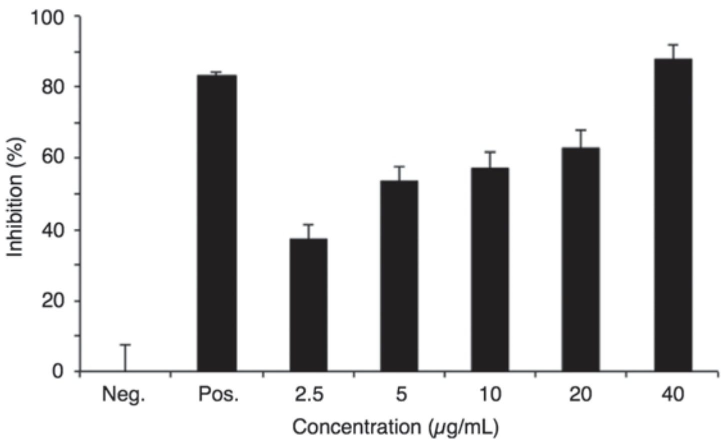

Fig. 2.3 Inhibition of advanced glycation end products by *V. myrtillus* leaf extract. Half maximal inhibitory concentration (IC_{50}) equals 17.35 µg·mL^{-1}

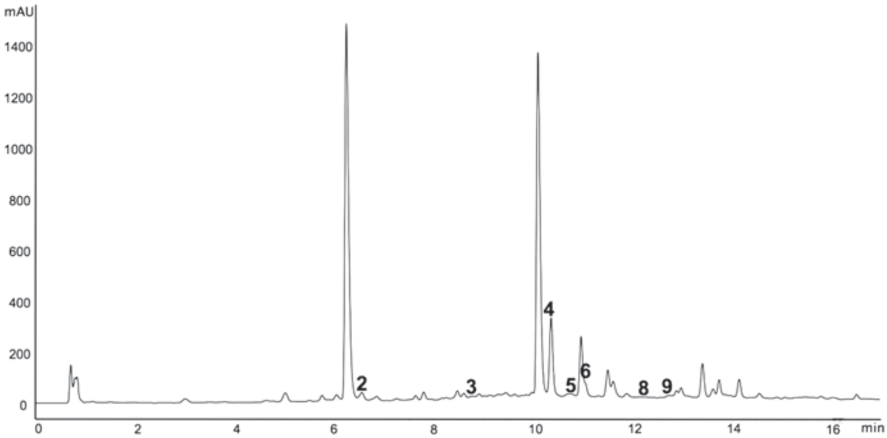

Fig. 2.4 HPLC chromatogram with DAD at 280 nm of *V. myrtillus* leaf sample. Numbers indicate presence of chlorogenic acid (*2*), para coumeric acid (*3*), taxifolin (*4*), quercetin-3-O-galactoside (*5*), quercetin-3-O-glucoside (*6*), quercetin-3-O-rhamnoside (*8*), and myricetin (*9*). Rutin and (+)-catechin were absent

2.3.4 HPLC–MS: V. myrtillus and V. vitis-idaea Markers

HPLC–MS analysis of *V. myrtillus* and *V. vitis-idaea* was compared with pure standards of (+)-catechin, chlorogenic acid, paracoumaric acid, taxifolin, quercetin-3-O-galactoside, quercetin-3-O-glucoside, rutin, quercetin-3-O-rhamnoside, and myricetin. Rutin was absent in both species (Fig. 2.4). *V. myrtillus* and *V. vitis-idaea* were separated based on the presence of (+)-catechin in *V. myrtillus* and quercetin 3-O-glucoside in *V. vitis-idaea*. All other metabolites identified were common to both species. Many of these compounds are good antioxidants and/or reported in other studies as active in antiglycation agents (McIntyre et al. 2009).

2.4 Conclusion

This study has identified species of interest for studies of complementary T2D medicines, while our analysis of *Vaccinium* spp. from Lukomir demonstrates the high activity of at least this genus and identifies its major constituents. There are both medicinal and many more food species utilized by the Lukomir Highlanders that remain to be studied. For example, phenolics and saponins likely play an important role in radical scavenging and cholesterol-binding activity in the Lukomir diet as they do in pastoral Maasai communities (Lindhorst 1998; Chapman et al. 1997; Johns et al. 1999). Eventually, clinical work is required to validate safe and effective use. Reinforcement or revival of traditional medicines and dietary plants with antidiabetic activity is especially important in these remote communities where modern health care is limited but traditional complementary medicines are abundant.

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