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Full Length Research Paper

Variation in essential oils to study the biodiversity in *Satureja montana L.*

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Albania has a very diverse climate from subtropical to continental and high relief variation, has a very rich flora. The Albanian flora comprises 3250 plant species or about 30% of the European flora, with about 30 endemic and 180 subendemic species. More than 300 species are aromatic or medicinal plants, which are very important economic natural resources. Further, they play an important role in everyday life. Of all these species, 68 species are endangered (EN). The employment of the new data analysis technique and statistical software provide a good support to conduct research in the field of medicinal and aromatic plants.

Key words: Albania, medicinal and aromatic plants, statistical software.

INTRODUCTION

Albania is one of the European countries which has a very rich flora because of several factors such as: a highly varied landscape including plains and mountain ranges, favorable climatic conditions ranging from coastal subtropical to inland continental climates, and geographical location in the Mediterranean region and in the Balkan Peninsula (Paparisto et al. 1988). The Albanian Flora has 3250 plant species, that is, about 30% of all known European plant species. These include about 30 species and 180 subspecies being endemic and subendemic (Paparisto et al., 1988). Albania's high biodiversity (species, ecosystems and habitats) provide good natural resources for medicinal and aromatic plants (MAPs) (Asllani, 2004). Folk preparation of about 300 medicinal plants are used for medicinal purposes and an estimated 10% of flora comprised medicinal and aromatic species (Vangjeli et al., 1995). Medicinal and aromatic plants have been used in traditional culture of Albania in folk medical practices since ancient times and much before the treatises of Paracelsius and Giambattista Porta of 18th century (Demiri, 1983), and these still play an important role in the ethnomedical concepts of the Albanians (Pieroni et al., 2005). Most MAPs belong to the family Lamiaceae such as *Salvia, Satureja, Sideritis, Thymus, Origanum, Micriomeria,* and these are used as herbal teas (Paparisto et al., 2003). Albania is one of the most important European exporting countries for MAPs. Albania exported about 10,000 tones of MAP dried raw material to the international market (Asllani, 2004). The whole county benefits from the essential oil industry. However, despite its exceptional richness in biodiversity, Albania is considered to have the highest rate of biodiversity loss in Europe. There is also considerable degradation of the environment. Thus, there is an urgent need to intervene in the germplasm safeguard and collection in Albania. But the budget is limited and the technology is limited, especially in data analysis area. Developing country like Albania is in urgent to find advanced, open-source (or with education discount), widely used and easy-to-use statistical software in order to optimizing the budget efficiency. After comparing several well known softwares, such as S-plus, SAS, SPSS, R, Sagemath and Matlab, we chose R, which is an open-source, to do the statistical analysis. The Mathematica is not the cheap. There are other cheaper or "free for public" software which can also do mathematical modeling, such as SIMILE and APSIM. But

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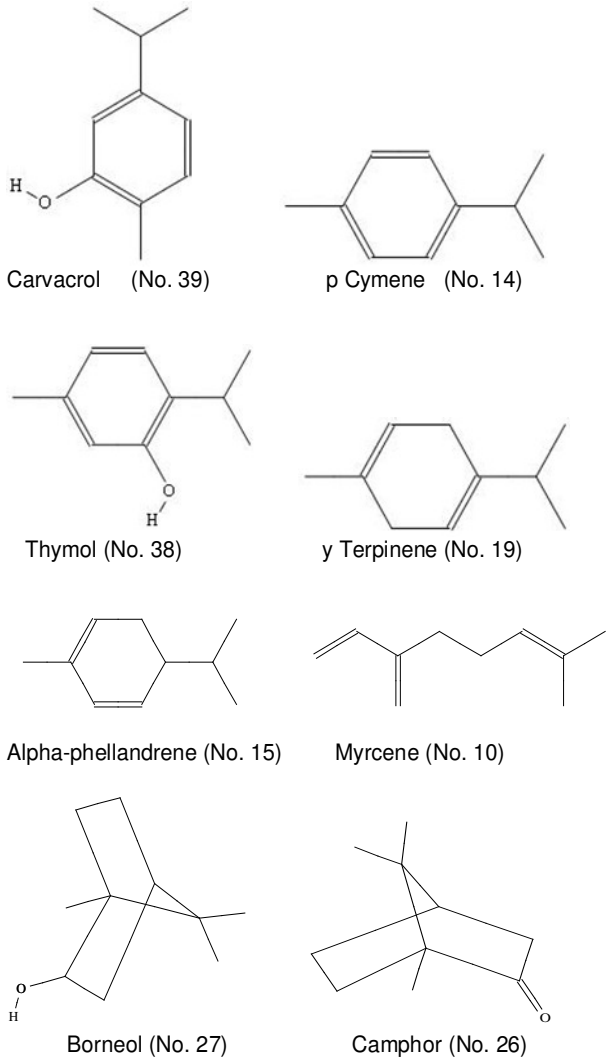

Figure 1. Chemical structure of some major compounds of medicinal and aromatic plants, generated by Mathematica7.

with the educational discount, Mathematica is the most efficient one. We also provide part of our code in the attachment to make our statistical analysis easily reproducible.

ISSUES

Chemical composition and use of *Satureja montana* essential oils

The use of essential oils has a potential interest in the pharmacological field and in food preservation technology for its antimicrobial effects. Several studies have been carried out on chemical characterization and the antibacterial properties of the essential oil of *Satureja montana* L. (Bellomaria and Valentini, 1985; Piccaglia et al., 1991; Slavkovska et al., 1997; Ibraliu et al., 2010). With regard to the presence of phenolic compounds, *S. montana* is known to possess antibacterial activity. Both of the essential oils, obtained by steam distillation, and the oleoresin are used in the food industry as flavoring agents, in the making of liqueurs and in perfumery (Guenther 1964; Capasso and Grandolini 1995; Piccaglia, 1998). Further, the essential oils have been used in the perfume industry, either alone or blended with other essential oils (Pepeljnjak et al., 1999).

Essential oils have antimicrobial and antidiarrheic activity because of the phenols in the oil. The main constituents of the essential oil in *S. montana* are Carvacrol and Thymol, as well as p-Cymene, β-Caryophyllene, Linalool and other terpenoids. Chemical structures of these compounds are shown in Figure 1. Ciani et al. (2000) examined the antimicrobial properties of *S. montana* essential oil on pathogenic and spoilage yeasts. The main components of essential oil were Thymol, p-Cymene, γ-Terpinene and Carvacrol. According to Lawrence (1979), the value of *S. montana* oil is in its high Carvacrol content and its fresh, spicy phenolic notes reminiscent of *Origanum vulgare* and *Thymus sp*. All these plant species contain essential oils with phenol compounds (Thymol and Carvacrol) as their major components. The content of Thymol and Carvacrol in *S. montana* is variable and depends on the origin and vegetative stage of the plant (Kustrak et al., 1996). The tea and extracts of this plant among many groups of natural compounds can contain free and glycoconjugated aroma compounds. Glycosidically bound volatiles were identified in many aromatic and non aromatic plants (Crouzet and Chassagne, 1999).

Due to this variation in usages of *S. montana*, we were interested to study the variability of populations collected from different sites in Albania, their essential oil content and composition. As a medicinal plant, S*. montana* has been used in folk medicine traditionally as a stimulant, stomachic, carminative, expectorant, antidiarrheic, and aphrodisiac. The essential oil has demonstrated antimicrobial and antidiarrheic activity because of the phenols in the oil (Ibraliu et al., 2010). High variability is evident among the population of *S. montana* growing in wild is known to possess high variability, even within a single population polymorphism and chemotype, especially those coming from distant habitats. There is large variation even within a single population genetic polymorphism (Ibraliu et al., 2010). The variability is reflected in morphologic characteristic such as the form and size of the calyx and corolla, calycal dents, size, form and color of the nutles, and particularly in the form, size and pubescence of the stems (Silic, 1979). Because these plants have been used in local spices and as a medicinal herb and also contain various biologically active constituents such as essential oil: triterpenes (Escudero et al., 1985), flavonoids (Thomas-Barberan et al., 1987), rosmarinic acid (Reschke, 1983) and the

strong phenolic character of its essential oil, which is reminiscent of the taste and fragrance of commercial *O. vulgare* and *Thymus* sp. oils. The main components of *Satureja boissieri* (Kurcuoglu et al., 2001) oil from Turkey were reported to be Carvacrol (40.8%) and c-Terpinene (26.4%). The main constituents of *Satureja brownei* (Rojas and Usubillaga, 2000) oil from Venezuela were found out to be Pulegone (64.3%) and Menthone (20.2%).

The main compound of *Satureja parvifolia* (Viturro et al., 2000) oil from Argentina was piperitone oxide and those of *Satureja boliviana* (Rojas and Usubillaga, 2000) oil were c-Terpinene, b-Caryophyllene and Germacrene D. Germacrene D has also been detected as the main compound of *Satureja coerulea* (Tumen et al., 1998a, b) oil from Turkey. The main components of *Satureja hortensis* (Baher et al., 2002), cultivated in Iran, were Carvacrol and c-Terpinene.

The essential oil of plant material obtained by steam or leaves distillation, produce oils rich in phenol compounds. Mild preservation technologies are becoming important in the modern food industry. In this context, spoilage yeasts may be a serious food contamination risk. The antimicrobial effects of essential oils on different microorganisms have been described. Until now, we have found only two published papers on variability of essential oil composition of this *S. montana* in Albania.

The germplasm safeguard and collection in Albania

Wild medicinal and aromatic plants are affected by the phenomena of genetic erosion. Due to its geographical characteristics, agricultural systems and methods, people's customs and historical factors, Albania can be considered of great interest for the germoplasm collection of wild medicinal and aromatic plants. The actual social and economic transformations of the area, without safeguarding programs to be carried out according to sustainability criteria, can produce a serious biodiversity decline. If over –exploitation is taking place these impacts may lead to decreasing plant population sizes, and genetic diversity, and finally to the extinction of the species. An additional impact in Albania has been the deregulation of state-controlled commerce resulting in an increase of collection MAP growing in wild.

SAMPLE PREPARATION

Isolation of the essential oil

The essential oil was isolated from dried leaf samples by hydro-distillation for 3 h using a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulphate and stored at 4°C. The oil solution (1%) in ethanol was used for chromatographic analysis. Preliminary GC–MS analysis showed the absence of 1-octanol as potential algycone of these species. The oil yield was determined by gravimetery. The component percentages were calculated as the mean value on column HP-20 M and HP-101 for duplicate analyses.

Analytical gas chromatography (GC/FID)

The GC/FID analysis of the oils was carried out on a Hewlett-Packard HP-5890 Series II GC apparatus equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (25 m, 0.32 mm, 0.52 µm film thickness) and fitted to flame ionisation detector (FID). Carrier gas (hydrogen) flow rate was 1 ml/min, split ratio was 1:30, injector temperature was 250°C, and detector temperature was 300°C, while column temperature was linearly programmed from 40 to 260°C (at rate of 4°/min). Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1 µl, split mode). Area percent reports, obtained as a result of standard processing of chromatograms, were used as base for the quantification purposes.

Gas chromatography/mass spectrometry (GC/MS)

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with column HP-5MS (30 m, 0.25 mm, 0.25 µm film thickness), using Hewlett-Packard HP G 1800C Series II GCD system.Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40 to 450. Sample solutions in ethanol (~1%) were injected by ALS (200 nl, split mode).

Identification of components

The compounds of the oil were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were deter-mined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1), compared to those from available literature (Adams, 2007), and used as additional tool to approve MS findings.

BENEFIT OF USING R AND MATHEMATICA7 TO ANALYZE THE CORRELATIONS AMONG ESSENTIAL OILS

R, invented by Robert Gentleman and Ross Ihaka, is widely used, well-known and open-source statistical software. It is currently maintained by the R core-development team. The advantages to use R is that it is not only an open source that people can use free, but also it has been integrated with good features, such as simple grammar rules and statistical functions for beginners, C and FORTRAN port for sophistic users, and literate programming technology for software project management Due to the fact that tens of thousands of researchers are sharing their software and packages there, one can easily do statistical analysis of their data. (R Development Core Team, 2006). Mathematica7, is the seventh generation of software Mathematical which is a very powerful computational software that is being widely used in natural sciences, engineering, finance and other scientific fields that require mathematical computation. It

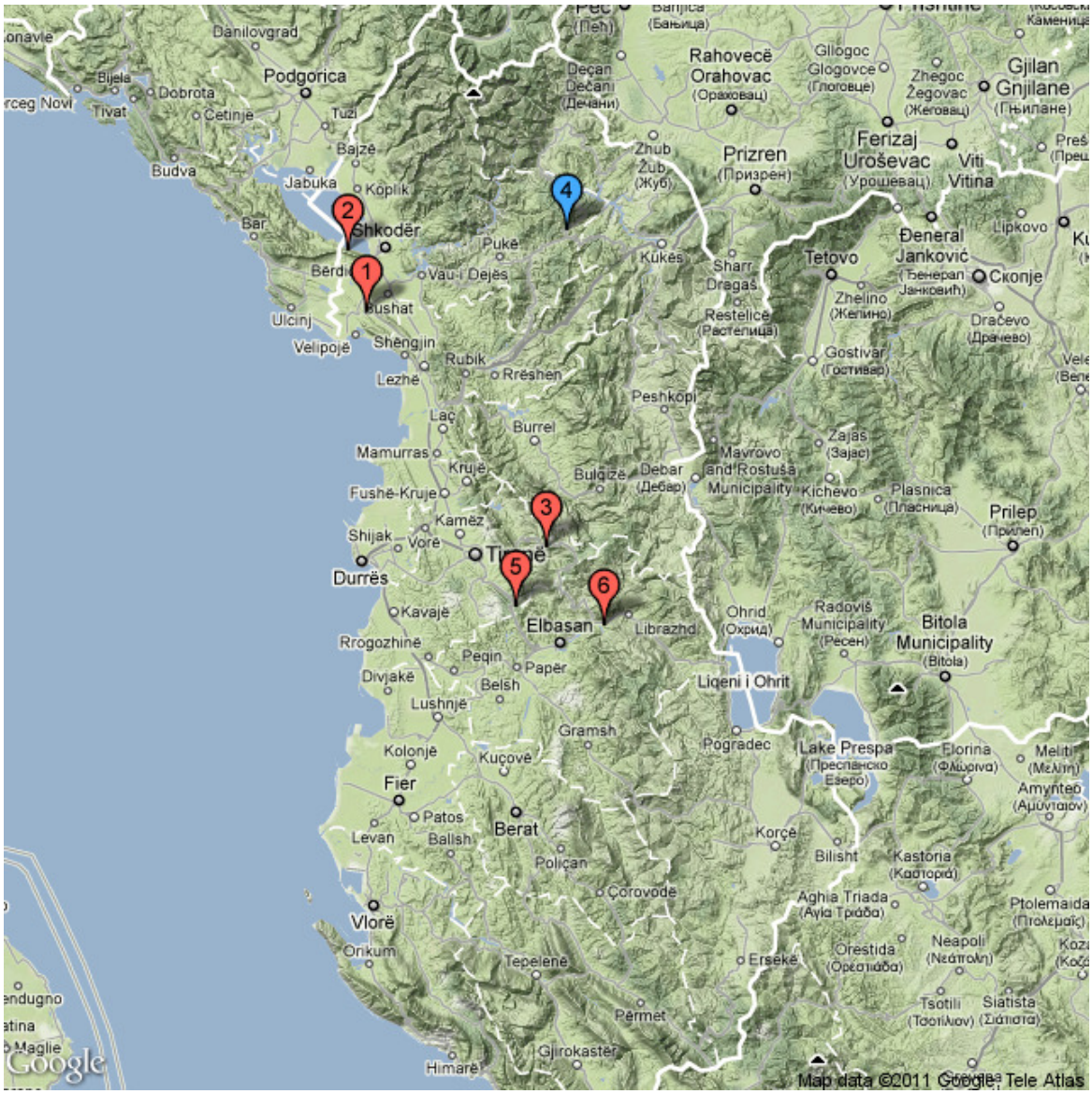

Figure 2a. Localization of populations at the study area with different climate, generated by R-package *Rgooglemap.*

was invented by Stephen Wolfram, who later opened Wolfram Research of Champaign, Illinois for further developing of the software. Its advantages are not only the high performance scientific computational abilities but also the visualized thinking-aid platform which covers almost all the area in science (Wolfram Research Inc, 2008). During our study, we first use R-googlemap package to prepare the map of location (Figure 2a). We verified that the oil compound is influenced by the geography information, such as attitude and climate. However, we find one location (P_4 from location 4) that could be an exception. Though, all six populations belonged to Mediterranean ecology, but P_4 was collected from a location quite away from the sea located in Mediterranean Continental Ecological Sub zone. It showed distinctly different composition of essential oil than other five populations. After carefully checking the googlemap, we notice that this one lies at the back of the slope facing the sea, which blocks the warm and humid wind from the ocean (Figure 2b). That niche has a special climatic condition which could provide special varieties of other plants too. Secondly, we use R-cluster package to do cluster analysis (CA) and principle components analysis (PCA). A classification of four chemical compounds in three species collected from 17 locations built (Ibraliu et al., 2011). Here we mainly presented another classification over 68 compounds in one species collected from six different locations (data presented in Tables 1 and 2), and the detailed R code is presented in the Appendix-code. We first use function *agnbb()* in

Figure 2b. Detailed map from area around location4 by *Rgooglemap*

Table 1. Basic characterization of the sites in Albania from where six *S. montana* populations were collected.

Population	Collection site	Altitude (masl)[a]	Latitude (N)	Longitude (E)	Exposition	Climatic Subzone
P_1	Kakariq, Lezhë	4	41°55'	19°28'	Plain field on the West of a North-South hill	Mediterranean
P_2	Zogaj, Shkodër	15	42°04	19°24'	Eastern slope of a North-South hill	Mediterranean
P_3	Dajt, Tiranë	1381	41°21'	20°03'	Eastern slope of a North-South mountain	Mediterranean
P_4	Qafë Mali, Pukë	800	42°7'	20°7'	Eastern slope of a North-South mountain	Continental
P_5	Qafë Kerrabë, Elbasan	750	41°12'	19°57'	Western slope of a North-South mountain	Mediterranean
P_6	Xibrakë, Librazhd	207	41°09'	20°14'	Western slope of a North-South hill	Mediterranean

[a] Altitude in meters above sea level

Table 2. The concentration and composition of the essential oil in six *S. montana* populations (P_1 to P_6) collected from six agro-ecolgically diverse sites in Albania.

Concentration/Compounds of the essential oil		RetentionIndex[a]	Population					
			P_1	P_2	P_3	P_4	P_5	P_6
Essential oil (% V/m)		**1.26**	**1.00**	**1.24**	**0.22**	**1.35**	**1.61**	
S/N	**Compounds**							
1	Trans-2-hexenal	873.8	-	-	-	0.13	-	-
2	Tricyclene	914.5	t[b]	t	t	0.21	0.08	-
3	α-Thujene	919.5	0.56	0.54	0.97	0.19	0.81	1.02
4	α-Pinene	926.8	1.05	0.74	0.90	1.56	1.35	1.11
5	Camphene	939.1	1.25	0.79	1.07	1.44	2.07	1.26
6	Thuja-2,4(10)-diene	955.6	-	-	-	0.03	-	-
7	Sabinene	969.3	-	-	-	3.06	-	-
8	β-Pinene	978.0	-	-	-	0.94	-	-
9	1-Octen-3-ol	982.8	0.81	0.97	0.60	-	1.12	0.95
10	β-Myrcene	990.7	0.71	0.70	0.98	7.81	0.82	0.92
11	3-Octanol	998.5	0.11	0.09	0.14	0.04	0.11	0.13
12	α-Phellandrene	1002.5	0.06	0.04	0.06	0.36	0.05	0.06
13	α-Terpinene	1010.4	0.80	1.26	1.35	0.04	1.03	1.16
14	p-Cymene	1019.3	14.79	15.31	13.34	1.13	16.22	17.40
15	β-Phellandrene	1026.6	0.55	0.56	0.55	6.09	0.70	0.78
16	1,8-Cineole	1028.6	0.66	0.49	0.70	0.12	1.07	0.60
17	Cis-β-ocimene	1044.8	-	-	-	2.38	-	-
18	Trans-β-ocimene	1052.0	-	-	-	0.76	-	-
19	γ-Terpinene	1056.0	4.80	4.87	8.86	0.31	5.25	5.16
20	Cis-sabinene hydrate	1065.9	0.46	3.20	0.47	0.16	0.46	6.17
21	α-Terpinolen	1082.6	0.19	0.41	0.14	0.21	0.18	0.19
22	Linalool	1099.2	2.45	3.63	0.35	1.63	0.42	0.79
23	1-Octen-3-yl acetate	1114.0	-	-	-	0.68	-	-
24	Cis-p-menth-2-en-1-ol	1116.9	0.07	0.25	t	0.18	0.06	0.10
25	Trans-pinocarveol	1134.7	0.12	0.24	0.09	0.28	0.14	0.18
26	Camphor	1137.8	0.14	0.22	0.11	6.86	0.20	4.12
27	Borneol	1160.6	4.27	2.96	3.82	9.64	6.68	1.35
28	Terpinen-4-ol	1173.2	1.13	3.42	0.95	0.80	1.34	0.16
29	p-Cymen-8-ol	1180.7	0.20	0.32	0.20	0.08	0.25	0.53
30	α-Terpineol	1185.4	0.54	0.54	0.33	0.36	0.55	t
31	Cis-dihydrocarvone	1191.2	0.12	0.18	0.09	0.36	0.15	t
32	Trans-dihydrocarvone	1198.0	t	0.15	t	0.07	t	t
33	Carvacrol methyl ether	1237.1	t	0.22		0.15	0.07	0.18
34	Carvone	1256.1	0.32	0.97	4.65	0.27	1.75	5.24
35	Geraniol	1267.4	0.12	4.64	t	-	t	t
36	Isobornyl acetate	1278.8	t	0.19	t	0.36	t	t
37	Neryl formate	1284.2	0.08	0.08	0.07	-	0.07	0.08
38	Thymol	1289.3	0.38	2.97	12.43	1.46	27.29	1.45
39	Carvacrol	1313.6	55.95	39.53	37.53	2.21	21.07	40.51
40	Eugenol	1324.2	0.43	0.39	0.14	t	0.17	t
41	α-Ylangene	1364.3	t	t	t	-	0.13	0.14
42	α-Copaene	1367.5	t	0.09	0.13	0.27	t	t
43	Carvacrol acetate	1369.5	t	0.14	t	-	t	t
44	Geranyl acetate	1380.6	t	0.07	0.17	-	0.19	0.14
45	β-Bourbonene	1381.6	-	-	-	1.68	-	-
46	Trans-β-caryophyllene	1410.8	3.37	3.53	3.46	10.79	2.86	2.53

Table 2. Contd.

47	β-Gurjunene (calarene)	1419.0	t	0.07	0.10	0.41	0.12	0.11
48	Aromadendrene	1431.2	0.33	0.38	0.33	0.33	0.60	0.65
49	α-Humulene	1443.1	0.12	0.13	0.14	0.77	0.11	0.10
50	Cis-cadina-1(6),4-diene	1460.5	-	-	-	0.20	-	0.26
51	γ-Muurolene	1469.7	0.11	0.10	0.26	0.32	0.29	-
52	Germacrene D	1473.7	t	0.08	0.50	10.44	0.09	0.12
53	Viridiflorene (ledene)	1487.5	0.16	0.24	-	-	0.50	0.60
54	Bicyclogermacrene	1499.2	-	-	-	4.09	-	0.94
55	β-Bisabolene	1501.9	0.57	0.37	1.68	-	1.06	-
56	γ-Cadinene	1506.4	t	0.10	0.20	0.89	0.18	0.16
57	δ-Cadinene	1516.0	0.16	0.18	0.39	1.60	0.38	0.33
58	α-Calacorene	1540.0	-	-	-	0.59	-	0.78
59	Elemol	1550.6	-	-	-	0.40	-	-
60	Spathulenol	1572.0	0.14	0.57	0.47	3.47	0.56	-
61	Caryophyllene oxide	1577.2	1.92	2.67	1.18	5.29	1.40	1.30
62	Salvial-4(14)-en-1-one	1595.7	-	-	-	0.49	-	t
63	Humulene epoxide II	1600.1	t	0.07	-	0.73	t	0.14
64	Caryophylla-4(12), 8(13)-dien-5α-ol	1628.3	t	0.15	0.10	-	t	t
65	γ-Eudesmol	1629.4	-	-	-	1.93	-	0.13-
66	α-Cadinol	1654.4	-	-	-	1.76	-	-
67	14-Hydroxy-9-epi-trans-caryophyllene	1663.8	t	0.17	-	0.43	t	t
68	Germacra-4(15),5, 10(14)-trien-1-α-ol	1687.2	-	-	-	0.76	-	-
Total number of compounds			38	50	41	58	44	42

[a] RI = retention index experimentally determined (AMDIS).
[b] t= trace less than 0.05%.

R-package cluster to analyze the data, the results are shown in Figures 3 and 4 with differentmetric "*euclidean*" and "*manhattan*". Both metrices suggest that Carvacrol (No. 39), Thymol (No. 38), p-Cymene (No. 14) and y-Terpinene (No. 19), which are the major compound of the essential oil (around 80%),can be classified as major compounds (The PCA of these four compounds is presented in Figure 5). We also find a second group, which includes seven different types of compounds (Compound No. 10, 15, 27, 26, 46, 52, 61) as shown from the CA with "*euclidean*" distance in Figure 3 and more different compounds (Compound No. 20, 60) from the "*manhattan*" distance in Figure 4. Ten compounds (Compound No. 10, 15, 27, 26, 46, 52, 61, 60, 54, 7) was identified by the PCA with major components equal to three (Figure 6). The total concentration of them is more than 10%. Especially in the plant from Location 4, the total concentration of them is 60.55%. Our interests are focused on Borneol (No. 27) , caryophyllene.oxide (No. 61) and caryophyllene (No. 46), whose concentration are more than 1% over all plants from six locations. We also have interests on three other compounds, Alpha-phellandrene (No. 15), Camphor (No. 26) and Myrcene (No. 10), which only have more than 1% concentration in plant from location 4. After looking at the chemical structures, six compounds (No. 10, 15, 26, 27, 46, 61) are finally chosen as the second group. Other compounds can be considered as minor group. P_4 from location 4, which have largest distance to others, is also identified (Figure 7). Then we use Mathematica7 to list all chemical symbols of all these 68 chemical compounds that is quite convenient with few commands (for example, ChemicalData ["Borneol"]) instead of looking for them in chemical handbook. Russo et al. (1998) has reported that the total amount of Thymol and Carvacrol are fixed and gave similar assumptions, we investigated the detail of this. By using Mathematica7 the result is visualized which makes the discovery easier and faster than Russo et al. (1998). A Mathematica7 code for discovering the biogenetic precursors over huge amounts of compounds is under development.

With this analysis we verified that the oil counts of Thymol and Carvacrol are negative correlated. We also verified that Thymol and p-Cymene are positive

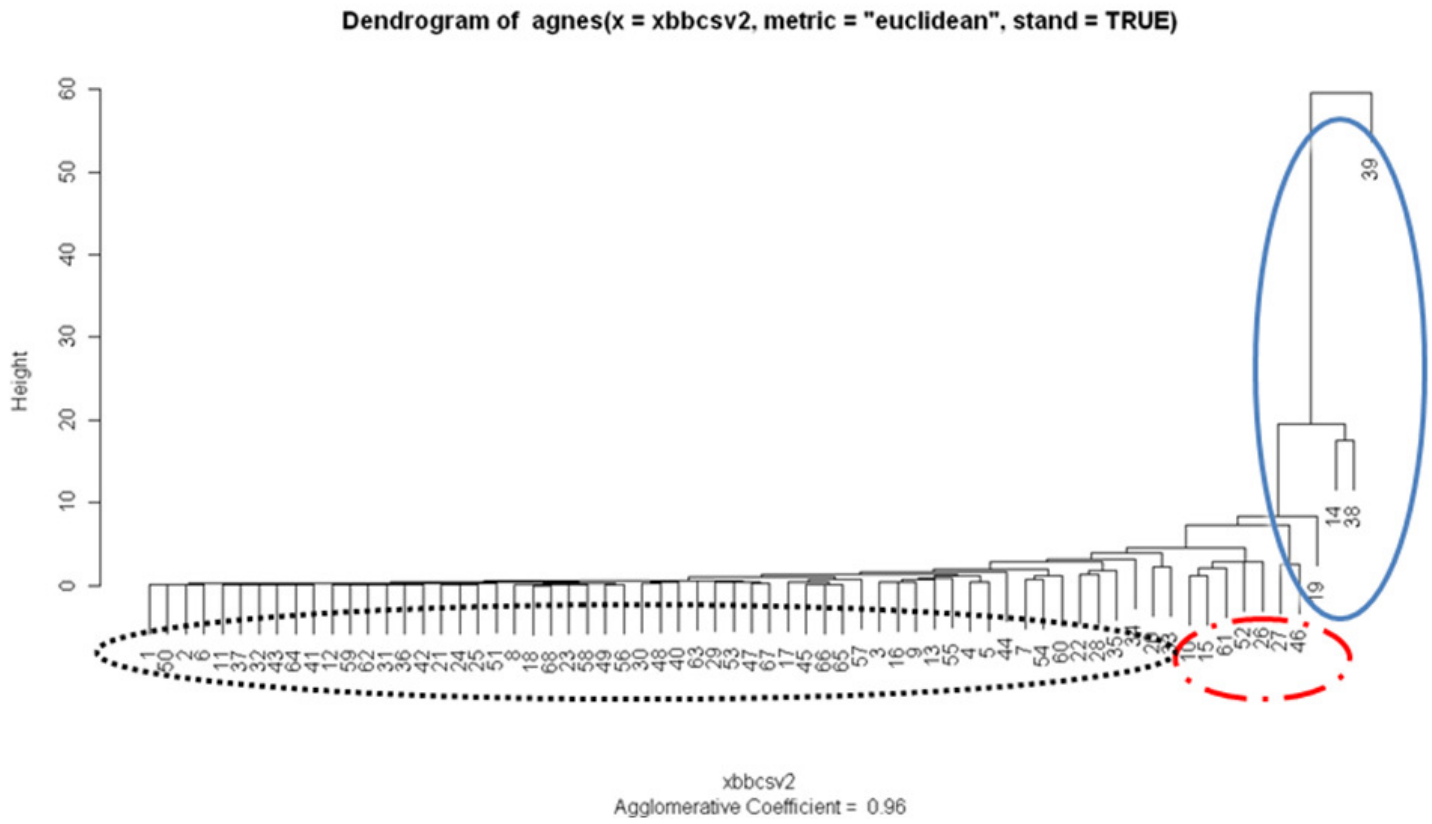

Figure 3. Cluster analysis of the 68 compounds, "*euclidean*" distances are root sum-of-squares of differences.

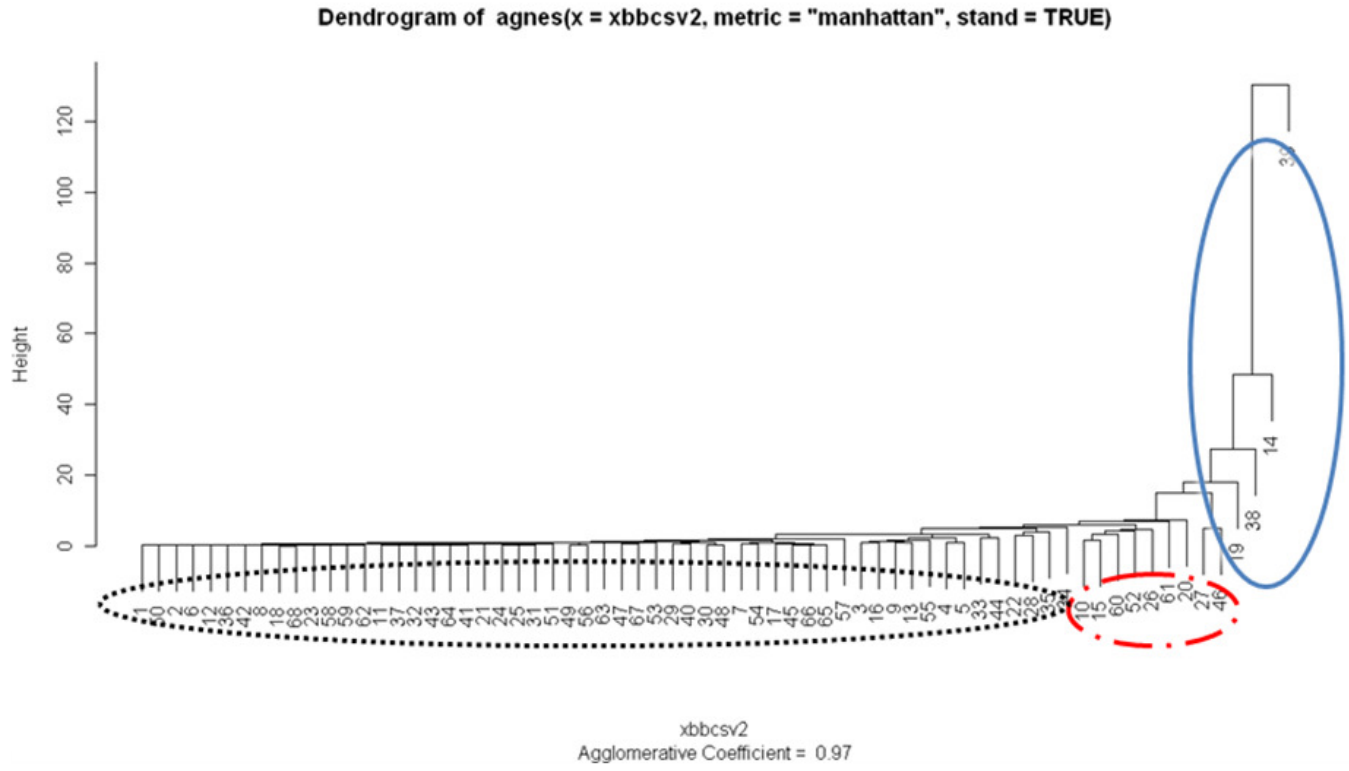

Figure 4. Cluster analysis of the 68 compounds. "*manhattan*" distances are the sum of absolute differences.

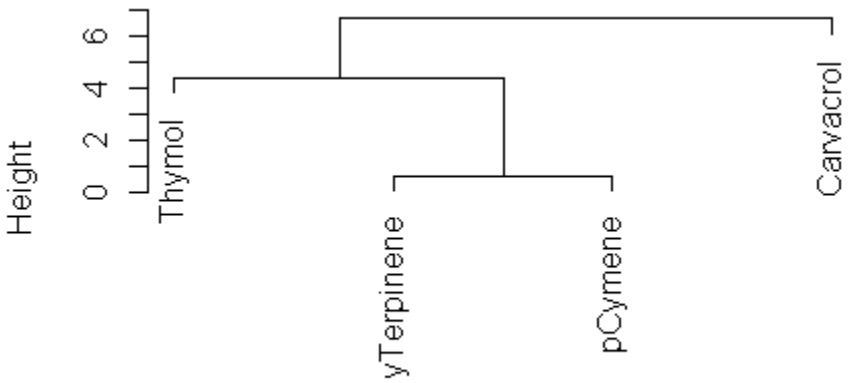

Figure 5. Cluster analysis according to major compounds of medicinal plants oils classification, generated by R.

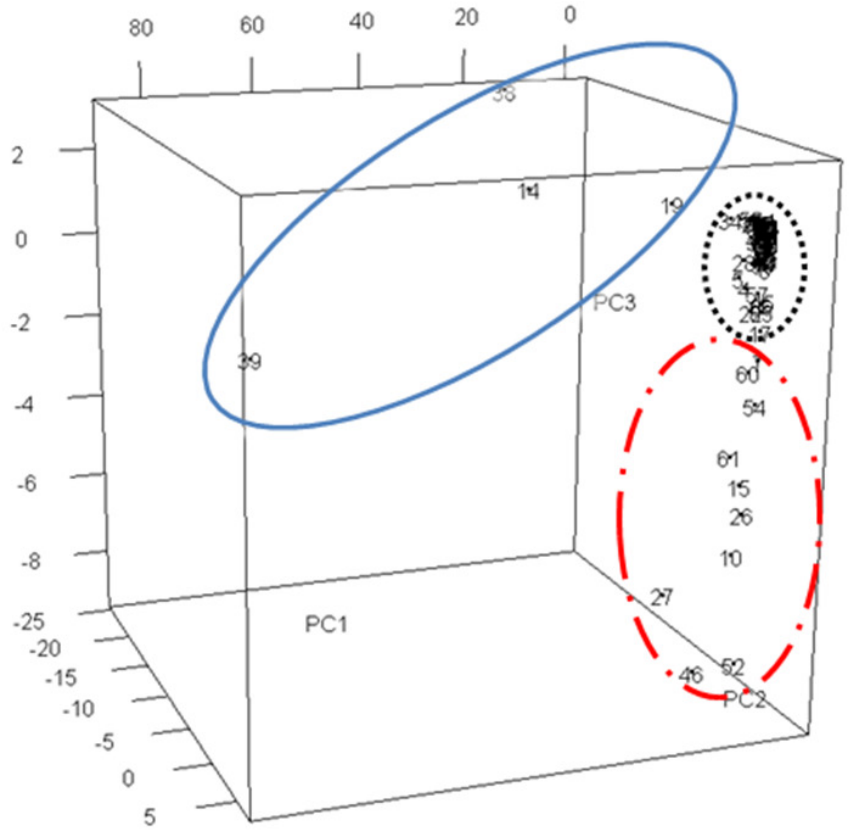

Figure 6. PCA of the oil data.

correlated. Last, by using Mathematica7 we analyzed all the chemical structures of all compounds and verified that p-Cymene and y-Terpinene are the biogenetic precursors of Thymol and Carvacrol. The metabolic pathways (y-Terpinene -> p-Cymene –> Thymol, y-Terpinene -> p-Cymene -> Carvacrol) are verified for Russo et al. (1998).

For the second group, Alpha-phellandrene, which is the isomer of y-Terpinene is found in plant from location 4. For certain unknown reasons, in that plant, Alpha-phellandrene are produced, instead of y-Terpinene. Then the whole pathway described by Russo et al. (1998) is blocked. The plant uses other pathway to produce more Borneol, Camphor, instead of Thymol and Carvacrol. Other interesting thing is for the Myrcene, Geraniol and Nerol, which are similar in structure and also have ten carbons as Borneol and Camphor. Croteau and Karp

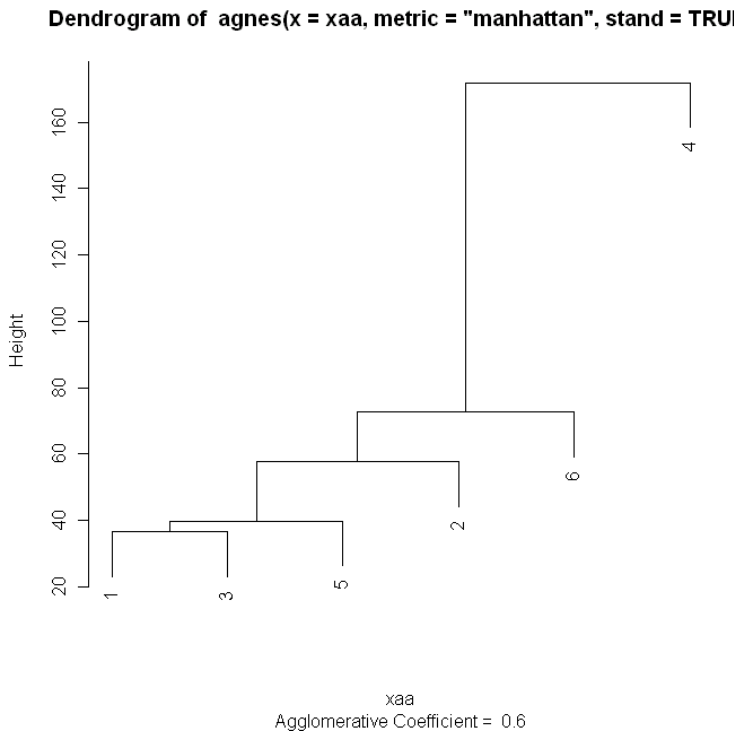

Figure 7. Cluster analysis of the 6 locations.

(1976) have found an enzyme which catalyzes neryl-pyrophosphate to Borneol in the presence of Mg++. We also found that Myrcene have a relatively high concentration in plant from location 4 and only traces in other plant and, while the situation is vice versa for y-Terpinene. Miceli et al. (2006) have also reported that the phenolic compounds and Myrcene have a negative correlation in *Thymbra capitata* (L.). So it suggests that there is a second pathway exists in *S. montana*, that is, Myrcene is the precursors of Borneol and Borneol can be easily oxidized into Camphor. A Mathematica7 code for discovering the biogenetic precursors over huge amounts of compounds is under development.

Due to the fact that the oil concentration is related to the climate conditions, we feel that the sunshine and the air moisture might be the influencing factors. But this could also be a specific genotype selected by environment.

DISCUSSION AND CONCLUSION

The implementation of R and Mathematica7 would give a synthetic pathway for the correlations among the compounds of essential oils. The pathways (y-Terpinene -> p-Cymene –> Thymol, y-Terpinene -> p-Cymene -> Carvacrol) given by Russo et al. (1998), who believes that the altitude would be the main factor that effects the chemical composition of essential oils, are verified. However, we found that the continental climate and attitude together are the main factors, which influence the pathway suggested by Russo et al. (1998). Percentage of compounds is different even for two samples collected from different locations having similar altitude. Based on that the phenolic compounds and Myrcene have a negative correlation, We also suggest another possible pathway (Myrcene --> Borneol -->. Camphor) in *S. montana*. This suggested hypothesis is made possible as a result of close cooperation between ecologist and mathematician. By “speaking” the open-source statistical language R, the procedure becomes very interesting and efficient.

Using R and Mathematica7 to estimate the biodiversities for protection of the biodiversity will be one of the objectives of our work in the future. From this point of view, the undertaking of new research and also preparing of the packages of this software will help the researcher in this area.

Here are still some questions that are open, such as, “is there a pathway in *S. montana* involving Myrcene, Caryophyllene.oxide and Caryophyllene?’’. Due to randomness, environmental effects and genetic properties, different statistical software or different criterion of classification in same statistical software might give similar but not identical results. With assistance of mathematical software, structure of the chemical molecules are built and the major and minor compounds are indentified with more chemical meaning full background.

But how to standardize the procedure is still an open question.

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Appendix-code

```
library(xlsReadWrite)
library(cluster)
library(labdsv)
library(rgl)
library(RgoogleMaps)

# set work folder and input of data from xls
setwd("K:\\mmm\\resources-hohenheim\\23-11-2009-alban")
xbbcsv2=read.csv(file="dataxbb.csv")

# can also input from remote

# Cluster analysis of the 68 compounds.
agnbb <- agnes(xbbcsv2, metric = "euclidean", stand = TRUE)
plot(agnbb) # PCA
 # returns the first 10 eigenvectors and loadings
 xbb.plot <- pca(xbbcsv2[,],dim=3)
# plot(xbb.plot, pch=c(1:6)+96)

plot3d(xbb.plot$scores)
text3d(xbb.plot$scores, texts=1:68)

# Analysis the minor group
minor=xbbcsv2[c(10,15,20,26,27,46,52,60,61),]

# google map


# here is a function to trans Sexagesimal into decimal.

sexa2dec<-function(x)
{
  theout1=floor(x)
  theout2=floor((x-theout1)*100)
  theout3=floor(((x-theout1)*100-theout2)*100)

  output=theout1+theout2/60+theout3/6000
  round(output,4)
}

# read the file for S. montana

xa=read.csv(file="simple.S. montana.csv",sep=";")

xb=xa
xb[,3]=sexa2dec(xb[,3]/10)
xb[,4]=sexa2dec(xb[,4]/10)
char=array(0,dim(xb)[1])
for (i in 1:dim(xb)[1])
{
char[i]=paste(i)
}

# plot like figure 1a in the manual

 markers=0

 for (i in 1:dim(xb)[1])
 {
  if (i==1)
  {

    if (xb[i,1]>=13)
    {
    markers=paste(xb[i,3],",",xb[i,4],",","blue",i,sep = "")
    }else
    {
    markers=paste(xb[i,3],",",xb[i,4],",","red",i,sep = "")
    }
  }else
{
     if (xb[i,1]==4)
    {
markers=paste(markers,"%7C",xb[i,3],",",xb[i,4],",","blue",i
,sep = "")



    }else
    {

markers=paste(markers,"%7C",xb[i,3],",",xb[i,4],",","red",i,
sep = "")
    }
     }
}

# show the map, should type y to get the file
GetMap(markers = markers, destfile = "S.Montana.png")
```