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Full Length Research Paper

Saikosaponins a and d roots concentration in five *Bupleurum* species from four mountains in China

L. Nyobe*, J-T. Zhang and S.T. Huang

College of Life Sciences, Beijing Normal University – Beijing 100875 P.R. China.

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***Bupleurum* species are widely distributed in China and its diversity is of a potential commercial incomes. In this study, five species of the genus *Bupleurum* were collected in four mountains in North China areas. The saikosaponins a and d (SSa and SSd) roots concentration were determined by high performance liquid chromatography (HPLC) and the results revealed that *B. smithii*, *B. sibiricum* and *B. scorzonerifolium* might be of great interest because of their high SSd content in which the –OH of C-23 is involved in their bioactivity. This study, besides being helpful for medicine quality control, provides an insight of the importance saikosaponins. Especially, SSd might play in the discovery of new drugs against bacterial or viral diseases such as HIV.**

Key words: *Bupleurum* spp., Saikosaponins a and d, roots content determination, HPLC.

INTRODUCTION

Scientific research into medicinal plants or herbs dates back thousands of years as cultures explored which plants were useful for what conditions and passed information on species, preparation, and dose through the ages. This longstanding use, as for Traditional Chinese Medicine (TCM) and European herbal medicine up to the present time, provides an invaluable database on the safety and efficacy of numerous species. Because of the complexity of chemical content and variety of bioactivities, herbal medicine offers the prospect of the kind of "built in" poly-pharmacology that is increasingly apparent for orthodox drugs (Perry and Howes, 2010).

Bupleurum species represent one of the most successful and widely used herbal drugs for treatment of many diseases over the past 2000 years. Thorough studies have been carried out on many species of this genus and have generated immense data about the chemical composition and corresponding biological activity of extracts and isolated secondary metabolites (Ashour and Wink, 2011). Nowadays, plenty of elements have been considered as important and, most probably, others will be recognized as essential in the future.

Investigation on the role of phytochemical constituents has become urgent and the determination of their major role, especially those known or suspected to reach man through the food chain or by other way. Studies on the concentrations of phytochemical elements in medicinal plants are important because knowledge on their composition and on the factors affecting them will probably lead to conclusions of economical value.

In most cases, the major and minor organic compounds in plant drugs responsible for the effect are known (Szentmihályi et al., 2006). In a pharmacological aspect, the phytochemical content in extracts is essential.

In 2009, there were about 33.2 million HIV-infected people in the world and AIDS is now a leading cause of death worldwide (Hunt, 2010). Buimovici-Klein et al. (1990) observed that saikosaponins have been found to inhibit human immunodeficiency virus in the test tube, yet it is unclear to what degree saikosaponins contributed to this effect. Piras et al. (1997) concluded that saponins also increased the efficacy of the standard anti-HIV drug *lamivudine* in the test tube.Human data are lacking on the benefit of saikosaponins in people with HIV infection.

In this study, the quantities of saikosaponins a and d of five species of the genus *Bupleurum* were estimated and the relationships between the phytochemical roots content and environment variables effects explored to postulate on the possible importance of these compounds

*Corresponding author. E-mail: lambertnyobe@yahoo.fr. Tel: +86 10 58807647. Fax: +86 58807721.

Table 1. Saikosaponins a and d average area from 1g of roots in Guandi Mountain.

Sites and species	Average quantity (g)		Saikosaponins average area		∑ area (∑a+d)
	Initial	Final	SSa	SSd	
GCF-1A	1	0.1726	300.2	284.2	584.4
GBF-1A	1	0.2100	255.3	252	507.3
GSL-1A	1	0.1932	300.7	307.9	608.6
GGL-1A	1	0.2252	251.7	243.6	495.3

GCF: Guandi Mountain Coniferous Forest; **GBF**: Guandi Mountain Broadleaves Forest; **GSL**: Guandi Mountain Shrubs Land; **GGL**: Guandi Mountain Grass Land; **1A**: Bupleurum bicaule.

in the elaboration of drugs with emphasis on diseases' such as HIV.

MATERIALS AND METHODS

The plants were collected from Guandi, Wutai, Xiao Wutai and Dongling mountains in China from June to September (2010). For each surveyed mountain, the plants were randomly taken in four distant environments to ensure that the resulting samples represent better the condition of each area. Identification of the collected *Bupleurum* species was confirmed by Prof. Liu Quanru expert in Plant Taxonomy at the College of Life Sciences of Beijing Normal University in China.

Roots samples analyses have been processed in the Laboratory of the Experimental Teaching Center of Life Sciences and Biotechnology at the College of Life Sciences of the Beijing Normal University as follows:

1 g of dried root was placed in a flask and 28.5 ml $CH3OH$ + 1.5 ml $NH4$ was added. This was heated in the Bain-Marie at 65°C for 1 h. The content was transferred in a new flask by filtering, and the operation with the first flask was repeated 3 times. 28.5 ml $CH3OH$ + 1.5 ml $NH4$ was added to the second flask and the solution was dehydrated with a Rotatory Evaporator (type RE 52AA) in a Bain-Marie at 40°C. 15 ml Stock ($CH3OH$ + roots extract) was carried in an HPLC system (Agilent Technology 1200 series Isocratic) equipped with a manual sample injector was carried out on a column C18 (150 mm x 4.6 mm x 5 µm). The gradient elution was constituted by a mobile phase of 600 ml ultrapure water and 400 ml acetonitrile, with a detection wave-length of 210 nm, flow rate of 1ml x min^{-1} and injection volume of 20 µl. The system was restored to its initial condition after 15 min. The recovery of saikosaponins a and d were 0.994 and 0.996 respectively. The reference saikosaponins a and d samples were provided by the Shanghai Biotechnology Ltd. CANOCO version 4.5 (Ter Braak and Smilauer, 2002) and IBM-SPSS version 19 programs were used to analyze relationships between environmental variables and saikosaponins.

RESULTS AND DISCUSSION

The quantities of saikosaponins a and d were estimated from the curves areas produced by HPLC of each saikosaponin.

Guandi mountain

Only *Bupleurum bicaule* was recorded. Table 1 shows the average quantities of saikosaponins a and d obtained from 1g of analyzed roots from different sites. The vertical analysis showed slight differences in saikosaponins a and d content in *Bupleurum bicaule* from the different environments. SSa/SSd ratios' were 1.06 for coniferous forest; 1.01 for broadleaves forest; 0.98 for shrubs land and 1.03 for grassland (Table 1).

The horizontal species analysis pointed out the relative highest difference in saikosaponins roots concentration between *B. bicaule* GSL (608.6) - *B. bicaule* GGL (495.3) with a ratio GSL/GGL = 1.23. The lowest concentration was recorded between *B. bicaule* GSL (608.6) - *B. bicaule* GCF (584.4) with a ratio GSL/ GCF = 1.04. *B. bicaule* Guandi Mountain appeared to be relatively rich in SSa than in SSd. *B. bicaule* SL and *B. bicaule* GL recorded respectively the highest and the lowest sum ∑a+d content, the difference observed between these two environments would suggest that *B. bicaule* might be a shade plant. The difference between SSa and SSd can be explained by the sensitivity of SSa accumulation processs to sun light, which maybe not favourable to its accrual (Figure 1).

Table 2 shows for inner site, SSa SL and SSa CF represented each 27% of the ∑a+d (55% and 53% respectively of the total phytochemical content) and also 27% each in the inter-sites SSa percentages. Inner site, SSd SL represented 28% of the ∑a+d (55% of the total phytochemical concentration) and also 28% of the inter-sites SSd percentages closely followed by SSd CF (26%). The sum ∑a+d represented 55% for GSL, 53% for GCF, and 46% for GBF and GGL for the entire phytochemical concentration inner site and the same order almost remained for the inter-sites classification (GSL 28%, GCF 27%, GBF 23% and GGL 22%).In our previous analysis, *B bicaule* SL appeared with the highest content in saikosaponins a and d. In term of individual molecule for a particular aim involving each of these molecules, the interest will not be the same. Chemically, SSa and SSd do not have the same structure and cannot have the same biological function. For an activity (example in pharmaceutical industry) requiring SSa and/or SSd, *B. bicaule* SL might be of a great interest.

Outcome

In Guandi Mountain, we found *B. Bicaule* favorable environment might be shrubs land in term of global sum

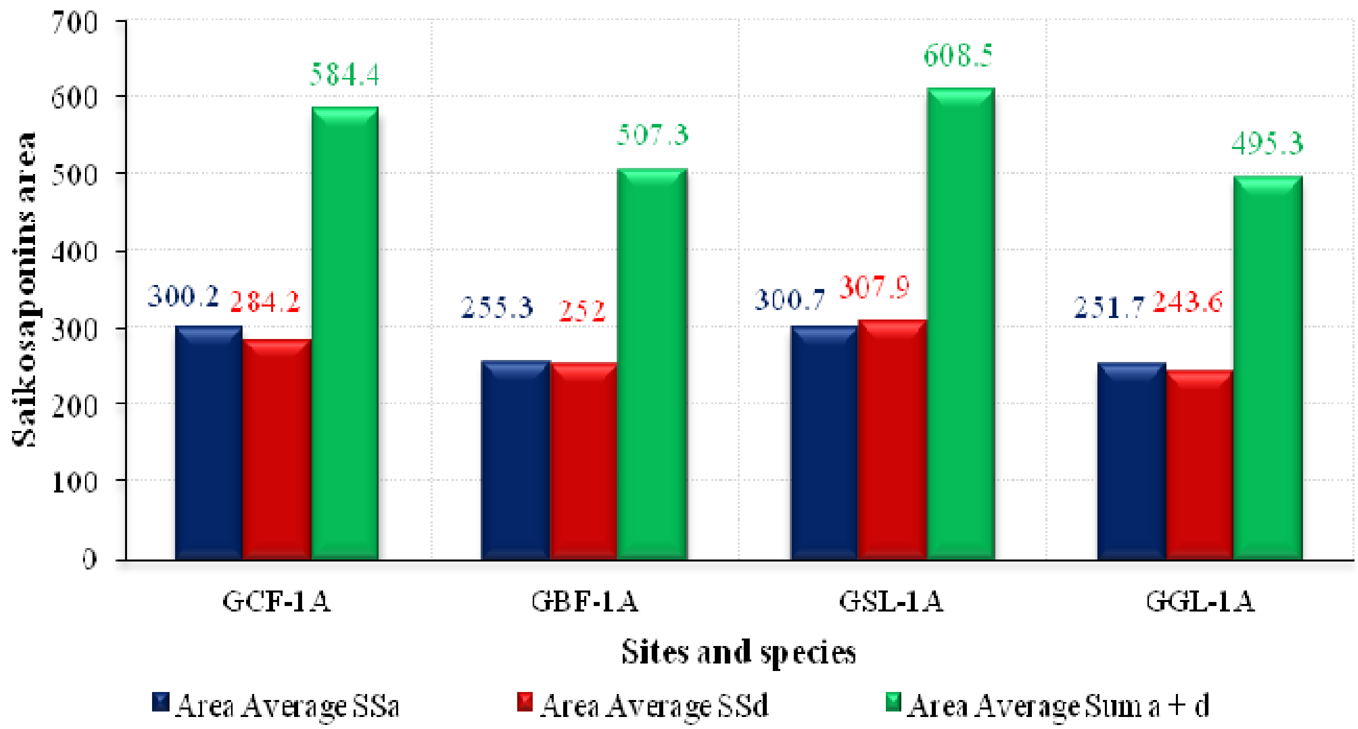

Figure 1. *Bupleurum bicaule* saikosaponins a and d distribution in Guandi Mountain. **GCF***: Guandi Mountain Coniferous Forest;* **GBF***: Guandi Mountain Broadleaves Forest;* **GSL***: Guandi Mountain Shrubs Land;* **GGL***: Guandi Mountain Grass Land;* **1A***: Bupleurum bicaule.*

Table 2. *Bupleurum bicaule* saikosaponins a and d average percentage in 1g of roots in Guandi Mountain.

Sites and species	Percentage (%)		
	SSa	SSd	∑a+d
GCF-1A	27	26	27
GBF-1A	23	23	23
GSL-1A	27	28	28
GGL-1A	23	23	22

GCF*: Guandi Mountain Coniferous Forest;* **GBF***: Guandi Mountain Broadleaves Forest;* **GSL***: Guandi Mountain Shrubs Land;* **GGL***: Guandi Mountain Grass land;* **1A***: Bupleurum bicaule.*

∑a+d saikosaponins roots concentration and also for a specific purpose aiming SSa and/or SSd.

Wutai mountain

Two species of *Bupleurum* genus were recorded: *Bupleurum bicaule* and *Bupleurum scorzonerifolium*. Table 3 summarizes the average quantities of saiko-saponins a and d obtained from 1 g of analyzed roots from each species.

The vertical analysis showed almost no difference in SSa and SSd concentration in *Bupleurum* spp. from coniferous and broadleaves forest, but remarkable variations appeared in shrubs and grass land where SSd/ SSa roots content were 1.43 and 1.53 respectively (Table 3).

B. bicaule horizontal species analysis showed the highest difference in saikosaponins roots concentration was between *B. bicaule* WGL (681.8) and *B. bicaule* WSL (501.5) with a ratio WGL = 1.36 WSL. The lowest saikosaponins roots concentration was between *B. bicaule* WGL (681.8) and *B. bicaule* WCF (675.8) with a ratio WGL = 1.01 WCF (Figure 2).

The horizontal inter-species analysis revealed that the highest saikosaponins roots concentration difference was between *B. scorzonerifolium* WBF (1159.1) – *B. bicaule* WSL (501.5) with a ratio of WBF = 2.31 WSL. The lowest

Table 3. Saikosaponins a and d average area from 1g of roots in Wutai Mountain.

Sites and species	Average quantity (g)		Saikosaponins average area		∑area (∑a+d)
	Initial	Final	SSa	SSd	
WCF-1A	1	0.1974	332.7	343.1	675.8
WBF-1B	1	0.1735	577.8	581.3	1159.1
WSL-1A	1	0.2232	206.6	294.9	501.5
WGL-1A	1	0.2221	270	411.8	681.8

WCF: Wutai Mountain Coniferous Forest; **WBF**: Wutai Mountain Broadleaves Forest; **WSL**: Wutai Mountain Shrubs Land; **WGL**: Wutai Mountain Grass Land; **1A**: *Bupleurum bicaule*; **1B**: *Bupleurum scorzonerifolium*.

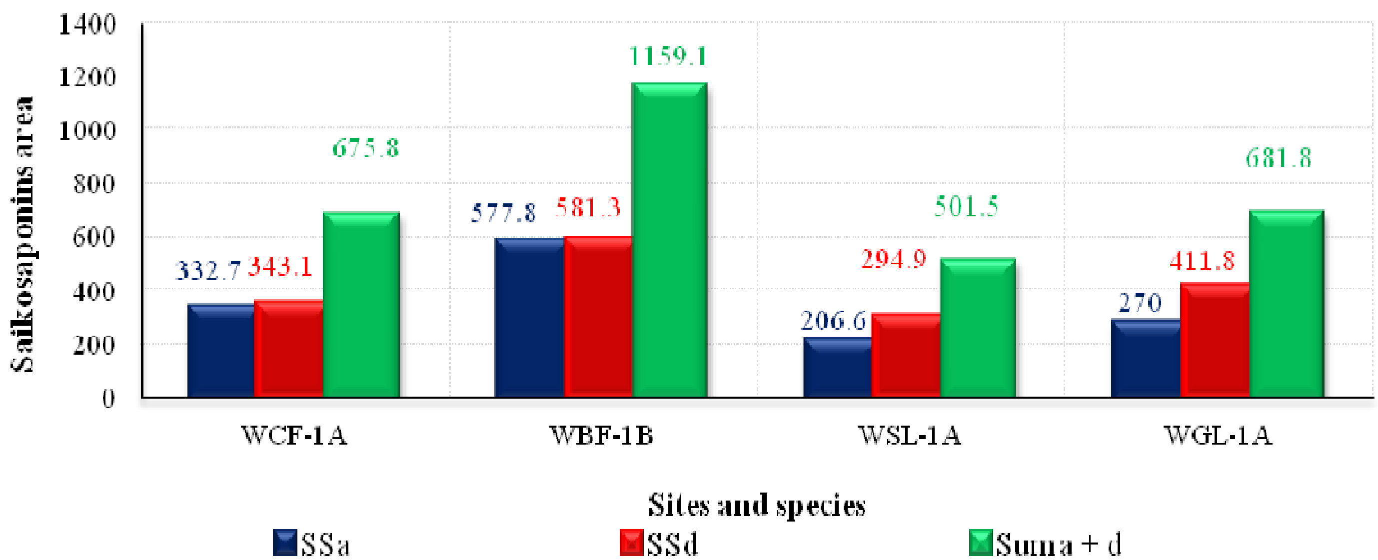

Figure 2. *Bupleurum* spp. saikosaponins a and d average area distribution in 1g of roots in Wutai Mountain. **WCF***: Wutai Mountain Coniferous Forest;* **WBF***: Wutai Mountain Broadleaves Forest;* **WSL***: Wutai Mountain Shrubs Land;* **WGL***: Wutai Mountain Grass Land;* **1A***: Bupleurum bicaule;* **1B***: Bupleurum scorzonerifolium.*

concentration was between *B. scorzonerifolium* WBF (1159.1) - *B. bicaule* WGL (681.8) with a ratio of WGL = 1.7 WSL. The ratio ∑a+d highest-lowest indicated *B. scorzonerifolium* WBF was 2.3 *B. bicaule* WSL in term of sum ∑a+d concentration and *B. scorzonerifolium* ∑a+d remained higher than *B.bicaule* ∑a+d whatever be the considered site (Figure 2).

The high content of saikosaponins in *B. scorzonerifolium* BF can be explained among many hypotheses by its preference for shade to sunny area. *B. bicaule* SL and GL received more direct sun radiations favorable for photosynthesis process leading eventually to high accumulation of saikosaponins, but we have noticed the ∑a+d *B. bicaule* CF was almost the same even more in some case than *B.bicaule* GL and SL. This constatation supported the previous one in Guandi Mountain in which *B. bicaule* was presumed to be shade oriented.

To explain the difference between SSa and SSd, we previously evoked the sensitivity of SSd accumulation processs to sun light, which maybe not favourable to its accrual, this hypothesis seemed valid for *B. scorzonerifolium* and relatively for *B.bicaule*. Parameters not measured in our survey like sunlight intensity and its duration, the exact age of our species or the undergone period of the survey among other might explain the observed fluctuation.

Table 4 depicts for inner site, SSa BF represented 42% of the ∑a+d (78% of the total phytochemical content) and also the inter-sites SSa percentages far followed by SSa CF (24%). Inner site, SSd BF represented 36% of the ∑a+d (78% of the total phytochemical concentration) and also inter-sites SSd percentages far followed by SSd GL (25%).

The sum ∑a+d represented WBF 78%, WCF 45%, WGL 44%, WSL 33% for the total phytochemical concentration inner site and WBF 38%, WGL 23%, WCF 22% and WSL 17% for the inter-sites classification. *B. scorzonerifolium* appeared with the highest content in saikosaponins a and d. For a specified activity requiring

Table 4. *Bupleurum* spp. saikosaponins a and d average percentage in 1g of roots in Wutai Mountain.

Sites and species	Percentage (%)		
	SSa	SSd	∑a+d
WCF-1A	24	21	22
WBF-1B	42	36	38
WSL-1A	15	18	17
WGL-1A	19	25	23

WCF: Wutai Mountain Coniferous Forest; **WBF**: Wutai Mountain Broadleaves Forest; **WSL**: Wutai Mountain Shrubs Land; **WGL**: Wutai Mountain Grass Land; **1A***: Bupleurum bicaule;* **1B***: Bupleurum scorzonerifolium.*

Table 5. Saikosaponins a and d average area from 1g of roots in Xiao Wutai Mountain.

Sites and species	Average quantity (g)		Saikosaponins average area		∑ area (∑a+d)
	Initial	Final	SSa	SSd	
XCF-1C	1	0.2466	379.9	314.7	694.6
XCF-1D	1	0.2410	387.9	810.5	1198.4
XBF-1C	1	0.1884	240.7	213	453.7
XBF-1D	0.72	0.1700	526.9	732.8	1259.7
XSL-1C	1	0.2151	249.8	267.5	517.3
XGL-1D	0.84	0.2609	354.2	605	959.2

XCF: Xiao Wutai Mountain Coniferous Forest; **XBF**: Xiao Wutai Mountain Broadleaves Forest; **XSL**: Xiao Wutai Mountain Shrubs Land; **XGL**: Xiao Wutai Mountain Grass Land; **1C***: Bupleurum sibiricum;* **1D***: Bupleurum smithi.i*

SSa and/or SSd, *B. scorzonerifolium* CF might be interesting (drug discovery).

Outcome

B. scorzonerifolium appeared only in the deciduous forest. We found *B. Bicaule* favorable environment might be evergreen and grassland in term of global sum ∑a+d saikosaponins roots concentration. *B. scorzonerifolium* favorable environment might be broadleaves forest in term of global sum ∑a+d saikosaponins roots content. *B. scorzonerifolium* SSa and SSd concentration remained higher than the concentration of *B. bicaule* whatever be the compared site.

Xiao Wutai mountain

Two *Bupleurum* species were recorded: *Bupleurum sibiricum* and *Bupleurum smithii*. Table 5 summarizes the saikosaponins a and d average quantities obtained from 1g (or less in some cases) of analyzed roots of each species.

The vertical analysis showed differences for both species and for each sites. *B. sibiricum* (1C) SSa/SSd was 1.21 in CF, 1.30 in BF and 0.93 in SL. It seemed that *B. sibiricum* roots were almost rich in SSa (particularly in XBF-1C) than in SSd (exception in the XSL-1C). *B. smithii* (1D) showed a predominance of SSd in the three sites of its presence. The ratios' SSa/SSd were evergreen forest 0.48, deciduous forest 0.72 and grassland 0.59 (Table 5).

The horizontal species analysis brought out *B. sibiricum* highest difference in saikosaponins roots content was between *B. sibiricum* XCF (694.602) and *B. sibiricum* XBF (453.7) with a ratio XCF = 1.53 XBF. The lowest saikosaponins roots concentration was between *B. sibiricum* XCF (694.6) and *B. sibiricum* XSL (517.3) with a ratio XCF = 1.34 XBF. *B. smithii* highest difference in saikosaponins roots concentration was between *B. smithii* XBF (1259.7) and *B. smithii* XGL (959.2) with a ratio XBF = 1.31 XGL. The lowest saikosaponins roots concentration was between *B. smithii* XBF (1259.714) and *B. smithii* XCF (1198.4) with a ratio XBF = 1.05 XCF (Figure 3).

The horizontal inter-species analysis showed the highest difference in saikosaponins roots concentration was between *B. smithii* XBF-1D (1259.7) and *B. sibiricum* XBF-1C (453.7) with a ratio XBF = 2.78 XBF. The lowest difference in saikosaponins roots content was between *B. smithii* XBF-1D (1259.7) and *B. smithii* XCF-1D (1198.4) with a ratio XBF-1D = 1.05 XCF-1D (Figure 3).

Sunlight could not be the possible factor to explain the saikosaponins roots concentration. In fact, *Bupleurum* spp. from broadleaves and coniferous forests received

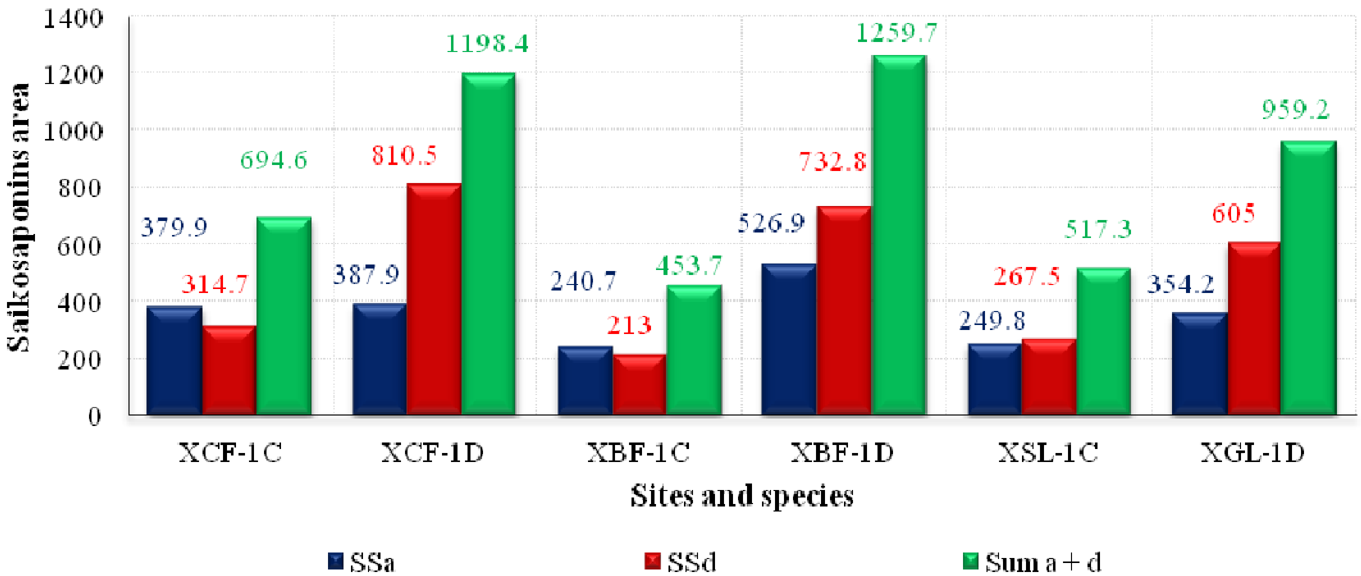

Figure 3. Bupleurum spp. saikosaponins a and d average distribution in 1g of roots in Xiao Wutai Mountain. **XCF**: Xiao Wutai Mountain Coniferous Forest; **XBF**: Xiao Wutai Mountain Broadleaves Forest; **XSL**: Xiao Wutai Mountain Shrubs Layer; **XGL**: Xiao Wutai Mountain Grass land; **1C**: *Bupleurum sibiricum;* **1D**: *Bupleurum smithii.*

Table 6. *Bupleurum* spp. saikosaponins a and d average percentage in 1g of roots in Xiao Wutai Mountain.

Sites and species	Percentage (%)		
	SSa	SSd	∑a+d
XCF-1C	18	11	14
XCF-1D	18	27	23
XBF-1C	11	7	9
XBF-1D	25	25	25
XSL-1C	12	9	10
XGL-1D	16	21	19

XCF: Xiao Wutai Mountain Coniferous Forest; **XBF**: Xiao Wutai Mountain Broadleaves Forest; **XSL**: Xiao Wutai Mountain Shrubs Layer; **XGL**: Xiao Wutai Mountain Grass land; **1C**: *Bupleurum sibiricum;* **1D**: *Bupleurum smithi.*

less direct sun radiations compared to *Bupleurum* spp. grass land. The difference in sum ∑a+d concentration showed saikosaponins roots accumulation might be inherent to other factor proper to the species; maybe the collected species might be shade oriented particularly *B. smithii*. Table 6 reveals inner site, SSa XBF-1D represented 25% of the ∑a+d (50% of the total phytochemical concentration) and also the inter-sites SSa percentages. Inner site, SSd XCF-1D represented 27% of the ∑a+d (45% of the total phytochemical concentration) and also the inter-sites SSd percentages closely followed by SSd XBF-1D 25% in inner and inter-sites distribution.

The sum ∑a+d represented XBF-1D 50%, XCF-1D 45%, XGL-1D 37%, XCF-1C 29%, XSL-1C 21% and XBF-1C 18% for the entire phytochemical concentration inner site; XBF-1D 25%, XCF-1D 23%, XGL-1D 19%, XCF-1C 14%, XSL-1C 10% and XBF-1C 9% for the inter-sites classification. *B. smithii* BF and CF (to some extent *B. smithii* GL) appeared with the highest content in saikosaponins a and d. For a specific activity targeting SSa and/or SSd, *B. smithii* whatever be the studied site will be interesting competed to *B. sibiricum*.

Outcome

In term of global sum ∑a+d saikosaponins roots concentration, *B. smithii* favorable environment might be deciduous, evergreen forest and grassland. *B. sibiricum* appeared coniferous forest oriented, even though this species can relatively grow in shrubs land and broadleaves forest. We also found *B. sibiricum* roots

Table 7. Saikosaponins a and d average area from 1g of roots in Dongling Mountain.

Sites and species	Average quantity (g)		Saikosaponins average area		∑ area (∑a+d)
	Initial	Final	SSa	SSd	
DCF-1E	1	0.1629	398.4	367.8	766.2
DBF-1E	1	0.1321	392.8	290.3	683.1
DSL-1E	1	0.1658	354.9	345	699.9
DGL-1E	1	0.1613	463	845.1	1308.1
DGL-1C	1	0.1318	195	478	673
DGL-1D	1	0.1914	348.3	294.4	642.7

DCF: Dongling Mountain Coniferous Forest; **DBF**: Dongling Mountain Broadleaves Forest; **DSL**: Dongling Mountain Shrubs Land; **DGL**: Dongling Mountain Grass Land; **1C**: *Bupleurum sibiricum;* **1D**: *Bupleurum smithii;* **1E**: *Bupleurum chinense.*

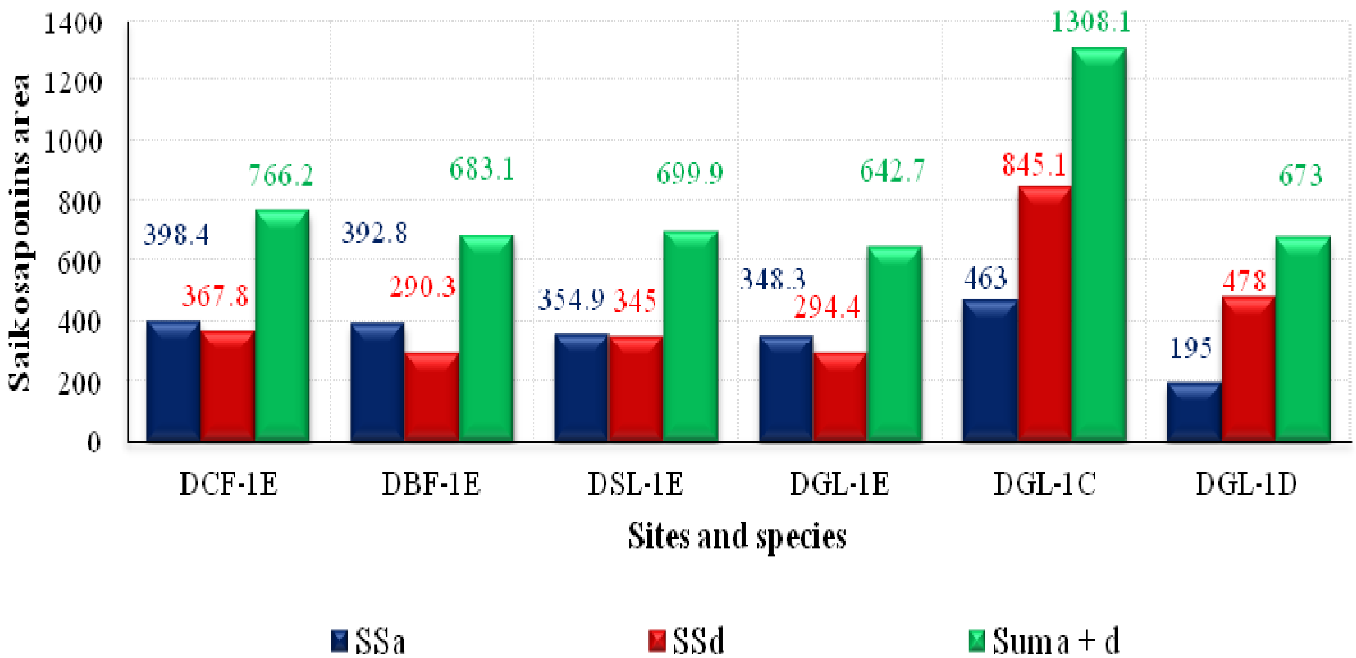

Figure 4. *Bupleurum* spp. saikosaponins a and d average distribution in 1g of roots in Dongling Mountain; **DCF**: Dongling Mountain Coniferous Forest. **DBF**: Dongling Mountain Broadleaves Forest; **DSL**: Dongling Mountain Shrubs Layer; **DGL**: Dongling Mountain Grass land; **1C**: Bupleurum sibiricum; **1D**: *Bupleurum smithii;* **1E**: *Bupleurum chinense.*

seemed rich in SSa than SSd while *B. smithii* roots contained more SSd than SSa.

Dongling Mountain

Bupleurum sibiricum, *Bupleurum smithii* and *Bupleurum chinense* were recorded. Table 7 summarizes the average saikosaponins a and d quantities extracted from 1 g of analyzed roots of each species.

B. chinense vertical analysis established difference between SSa and SSd roots quantities. SSa roots concentration remained predominant in the four surveyed sites. The ratios' SSa/SSd were evergreen forest 1.08, deciduous forest 1.35, shrubs land 1.03 and grassland 0.55. *B. sibiricum* and *B. smithii*, were only present in the grassland and the SSa/SSd ratios' were 0.41 and 1.18 respectively (Table 7).

The horizontal species analysis depicted *B. chinense* highest difference in saikosaponins quantity was between *B. chinense* DCF-1E (766.2) and *B. chinense* DGL-1E (642.7) with a ratio DCF = 1.19 DGL. The lowest saikosaponins roots concentration was between *B. chinense* DCF-1E (766.2) and *B. chinense* DSL-1E (699.9) with a ratio DCF = 1.09 DSL (Figure 4).

The horizontal inter-species analysis indicated the highest difference in saikosaponins roots concentration was between *B. sibiricum* DGL-1C (1308.1) and *B. chinense* DGL-1E (642.7) with a ratio DGL = 2.04 DBF.

Table 8. *Bupleurum* spp. saikosaponins a and d average percentage in 1g of roots in Dongling Mountain.

Sites and species	Percentage (%)		
	SSa	SSd	∑a+d
DCF-1E	19	14	16
DBF-1E	18	11	14
DSL-1E	16	13	15
DGL-1E	16	11	14
DGL-1C	22	33	27
DGL-1D	9	18	14

DCF: Dongling Mountain Coniferous Forest; **DBF**: Dongling Mountain Broadleaves Forest; **DSL**: Dongling Mountain Shrubs Land; **DGL**: Dongling Mountain Grass Land; **1C**: *Bupleurum sibiricum;* **1D**: *Bupleurum smithii;* **1E**: *Bupleurum chinense.*

The lowest difference in saikosaponins roots quantity was between *B.sibirum* DGL-1C (1308.1) and *B. chinense* DCF-1E (766.2) with a ratio DGL = 1.71 DCF (Figure 4).

The high saikosaponins roots concentration of *B. sibiricum* in the relative opened air site like shrubs land might result from its high exposure to direct sun radiations among different biotic and abiotic factors.

Table 8 represents a comparative percentage of SSa and SSd inner and inter-sites. Inner site, SSa DGL-1C represented 22% of the ∑a+d (55% of the total phytochemical content) and also the inter-sites SSa percentages. Inner site, SSd DGL-1C represented 33% of the ∑a+d (55% of the total phytochemical concentration) and also the inter-sites SSd percentages.
The sum ∑a+d represented DGL-1C 55%, DCF-1E 33%, DSL-1E 29%, DBF-1E 29%, DGL-1D 27% and DGL-1E 27% for the entire phytochemical concentration inner site; DGL-1C 27%, DCF-1E 16%, DSL-1E 15%, DGL-1D 14%, DGL-1E 14% and DBF-1E 14% for the inter-sites classification.

The three species relatively appeared with high saikosaponins a and d roots content. For a specific activity targeting SSa and/or SSd, *B. smithii* might be of an important sake. Beside *B. smithii*, *B chinense* and *B. sibiricum* can be the prospective source for SSa and SSd respectively.

Outcome

In Dongling Mountain, three species were recorded: *Bupleurum sibiricum*, *Bupleurum smithii* and *Bupleurum chinense*. *Bupleurum chinense* appeared in the whole extent of the four sites; while *B. sibiricum* and *B. smithii* appeared specific to grassland. In terms of total sum ∑a+d saikosaponins roots concentration, we found *B. smithii* favorable environment might be grassland. *B. chinense* favorable environment might be coniferous forest closely followed by deciduous forest, shrubs land and grass land. We also found *B. smithii* roots and *B. sibiricum* roots contained more SSd than SSa when *B. chinense* contained more SSa than SSd.

DISCUSSION

In a global view, our results showed in term of SSa and SSd average concentration of analyzed roots, the five recorded *Bupleurum* species can be classified in two ways as follows:
(i) The general average classification by site and species: *B. scorzonerifolium* (1159.1), *B. smithii* (1022.5), *B. sibiricum* (743.4), *B. chinense* (6978), *B. bicaule* (579.2).
(ii) The general average of average classification by site and species: *B. scorzonerifolium* (1159.1), *B. sibiricum* (931.7), *B. smithii* (906), *B. chinense* (698), *B. bicaule* (584.3).

The above classifications partially corroborated Pan (2006) results. In a decreasing manner (exclude *B. sibiricum*), Pan (2006) found the following ranking in term of SSa and SSd roots quantity: *B. chinense*, *B. smithii*, *B. scorzonerifolium* and *B. bicaule* even if the results were presented in term of percentage without precision about the initial and the final quantities.

Considered individually saikosaponins a and d quantities, the previous classification pointed out:
(iii) The general average classification by site and species:
SSa - *B. scorzonerifolium* (577.8), *B. chinense* (391.5), *B. smithii* (366), *B. sibiricum* (333.4), and *B. bicaule* (273.9).
SSd - *B. smithii* (656.6), *B. scorzonerifolium* (581.3), *B. chinense* (428.5), *B. sibiricum* (410.1) and *B. bicaule* (305.3).
(iv) The general average of average classification by site and species:
SSa - *B. scorzonerifolium* (577.8), *B. sibiricum* (419.8), *B. chinense* (391.5), *B. bicaule* (269.9) and, *B. smithii* (252).
SSd - *B. sibiricum* (700.1), *B. scorzonerifolium* (581.3), *B. smithii* (537.5), *B. chinense* (428.5) and, *B. bicaule* (380.9).

Our results also pointed out *sensu-lato* whatever be the

Table 9. Correlation coefficients between SSa, SSd, Sum ∑a+d quantity and Altitude.

Root concentration	Similarity matrix correlations				
	Correlation	**SSa**	**SSd**	**Sumad**	**Altitude**
SSa	Pearson Correlation	**1**			
	Sig. (2-tailed)				
	N	20			
SSd	Pearson Correlation	0.629**	**1**		
	Sig. (2-tailed)	0.003			
	N	20	20		
Sumad	Pearson Correlation	0.828**	0.956**	**1**	
	Sig. (2-tailed)	0.000	0.000		
	N	20	20	20	
Altitude	Pearson Correlation	-0.326	0.133	-0.027	**1**
	Sig. (2-tailed)	0.161	0.577	0.910	
	N	20	20	20	20

** Correlation is significant at the 0.01 level (2-tailed).

classification and the considered *Bupleurum* species, SSd roots content remained higher than SSa, except for *B. scorzonerifolium* SSa and SSd were almost the same. Our results looked partly in accordance with Pan (2006) who found SSa lower than SSd for *B. chinense* and *B. smithii*, SSa higher than SSd for *B. scorzonerifolium* and SSa equal to SSd for *B. bicaule*. Naturally *sensu-stricto* the following could not be considered as definitive because many factors biotics and abiotics influenced the saikosaponins variation in the different parts of the plant and also the genetic properties inner to each species are liable to explain the observed difference.

Our results show saikosaponins roots quantities tended to be low in opened areas where direct radiations are high (shrubs and grassland) compared to evergreen and deciduous forest.

Our results are not supportive with Huang et al. (2009) who found *Bupleurum* species in Dongling Mountain with the specific case of *B. chinense*, showed high saikosaponins roots contents in meadow environment and low saikosaponins roots contents in understory and brushy environment.Our results depicted in an increase manner *B. chinense* saikosaponins a and d contents seemed naturally lower than *B. scorzonerifolium* and *B. smithii* in accordance with Du (1991) and Li et al. (2006) for whom *B. scorzonerifolium* saikosaponins a and d is higher than *B. smithii*.

Our study compared to previous survey pointed out *B. chinense* from North China equated to South China areas SSa ≥ SSd in accordance with Lin et al. (2004, 2002). We also found *B. chinense* SSa seemed to be higher than SSd in North China areas in accordance with Min and Song (2010). These latter authors found SSa remained higher than SSd whether be *B. chinense* wild or cultivated.

Relationship between ecological factors and saikosaponins roots contents

To assess the correlation extent between saikosaponins a and d individually, their sum and four major ecological factors, we used the Bivariate Analysis (Pearson Correlation) and the One-way Anova of the program IBM-SPSS Statistics 19. The tables issued are shown below.

Correlation between SSa, SSd, Sum ∑a+d quantity and altitude

Credit given to Buda and Jarynowski (2010) on the Pearson's correlation size interpretation, the similarity matrix showed the pairs SSa – SSd (≈ 0.63), SSa – Sum ∑a+d (≈ 0.83) and SSd - Sum ∑a+d (≈ 0.96) are strongly correlated positevely.

The positive correlation implied that higher SSa (SSd) roots quantity tended to go with higher SSd and Sum ∑a+d roots quantities.

The similarity matrix (Table 9) outlined small negative correlation between Altitude – SSa (≈ -0.33); none correlation between Altitude – SSd (≈ -0.03) and small correlation between Altitude - Sum ∑a+d (0.13). This revealed higher was the environmental factor, lower was the SSa and SSd, but highher was the Sum ∑a+d roots content. The ANOVA (Table 10) showed significant difference between Altitude and SSa, SSd, Sum ∑a+d

Table 10. ANOVA of SSa, SSd, Sum ∑a+d quantity and Altitude.

Root concentration	ANOVA					
	Between/within group	Sum of Squares	df	Mean Square	F	Significance
SSa	Between Groups	187405	18	10411	1.075	0.652**
	Within Groups	9684	1	9684		
	Total	197089	19			
SSd	Between Groups	718944	18	39941	7.723	0.277**
	Within Groups	5171	1	5171		
	Total	724116	19			
Sumad	Between Groups	1367062	18	75948	2.618	0.456**
	Within Groups	29010	1	290010		
	Total	1396071	19			

**Correlation is significant at the 0.01 level

Table 11. Correlation coefficients between SSa, SSd, Sum ∑a+d quantity and pH.

Root concentration	Similarity matrix correlation				
	Correlation	SSa	SSd Sumad	Sumad	pH
SSa	Pearson Correlation	**1**			
	Sig. (2-tailed)				
	N	20			
SSd	Pearson Correlation	0.629**	**1**		
	Sig. (2-tailed)	0.003			
	N	20	20		
Sumad	Pearson Correlation	0.828**	0.956**	**1**	
	Sig. (2-tailed)	0.000	0.000		
	N	20	20	20	
pH	Pearson Correlation	-0.125	-0.305	-0.266	**1**
	Sig. (2-tailed)	0.599	0.192	0.256	
	N	20	20	20	20

** Correlation is significant at the 0.01 level (2-tailed).

supporting the hypothesis of saikosaponins roots content variation with elevation of the Pearson Correlation Test.

Correlation between SSa, SSd, Sum ∑a+d quantity and pH

The similarity matrix (Table 11) revealed small negative correlation between pH – SSa (≈ -0.13), medium negative correlation between pH – SSd (≈ -0.31) and small negative correlation between pH - Sum ∑a+d (≈ -0.30). Negative correlation pH SSa/ SSd/ Sum ∑a+d indicated that higher was the pH lower were SSa, SSd and Sum ∑a+d roots content. The ANOVA (Table 12) pointed non-significant difference between pH and SSa, SSd and Sum ∑a+d indicating the independency of saikosaponins roots accumulation on tributary to pH and supporting the hypothesis of saikosaponins roots content weak fluctuations with pH of the Pearson Correlation.

Correlation between SSa, SSd, Sum ∑a+d quantity and Vegetation

The similarity matrix (Table 13) brought out small negative correlation between Vegetation - SSa, none correlation between Vegetation – SSd (0.07) and Vegetation - Sum ∑a+d (-0.06). This depicted small

Table 12. ANOVA of SSa, SSd, Sum ∑a+d quantity and pH.

Root concentration	ANOVA					
	Between/within group	Sum of Squares	df	Mean Square	F	Significance
SSa	Between Groups	87852	13	6758	0.371	0.937^{ns}
	Within Groups	109237	6	18206		
	Total	197089	19			
SSd	Between Groups	294129	13	22625	0.316	0.961^{ns}
	Within Groups	429987	6	71664		
	Total	724115.870	19			
Sumad	Between Groups	621289	13	47791	0.370	0.937^{ns}
	Within Groups	774782	6	129130		
	Total	1396071	19			

ns:Correlation non- significant at the 0.01 level.

Table 13. Correlation coefficients between SSa, SSd, Sum ∑a+d quantity and Vegetation

Root concentration	Similarity matrix correlations				
	Correlation	SSa	SSd	Sumad	Vegetation
SSa	Pearson Correlation	1			
	Sig. (2-tailed)				
	N	20			
SSd	Pearson Correlation	0.629**	1		
	Sig. (2-tailed)	0.003			
	N	20	20		
Sumad	Pearson Correlation	0.828**	0.956**	1	
	Sig. (2-tailed)	0.000	0.000		
	N	20	20	20	
Vegetation	Pearson Correlation	-0.284	0.067	-0.059	1
	Sig. (2-tailed)	0.224	0.780	0.806	
	N	20	20	20	20

** Correlation is significant at the 0.01 level (2-tailed).

changes of saikosaponins roots content with the vegetation type. SSa roots quantity looked variable with vegetation compared to SSd. The ANOVA (Table 14) showed significant difference only between Vegetation and SSa, backing the hypothesis of saikosaponins roots content variation with vegetation of the Pearson Correlation Test.

Correlation between SSa, SSd, Sum ∑a+d quantity and Slope

The similarity matrix (Table 15) pointed out none correlation between Slope - SSa, Slope – SSd and Slope - Sum ∑a+d. This outlined no fluctuation of saikosaponins roots content with the Slope changes. The ANOVA (Table 16) presented a non-significant variation between Slope – SSa, SSd and Sum ∑a+d in accordance with the hypothesis of saikosaponins roots content non dependent to variable Slope of the Pearson Correlation Test.

Conclusion

Early studies on the genus *Bupleurum* had focused only on the traditional uses of the plants in the treatment of inflammatory disorders and infectious diseases. After chemical profiling, several groups of secondary metabo-

Table 14. ANOVA of SSa, SSd, Sum ∑a+d quantity and Vegetation.

Root concentration	ANOVA					
	Between/within group	Sum of squares	df	Mean Square	F	Significance
SSa	Between Groups	38747	3	12916	1.305	0.307**
	Within Groups	158342	16	9896		
	Total	197089	19			
SSd	Between Groups	74911	3	24970	0.615	0.615 ns
	Within Groups	649205	16	40575		
	Total	724116	19			
Sumad	Between Groups	149526	3	49842	0.640	0.600 ans
	Within Groups	1246545	16	77909		
	Total	1396071	19			

Threshold value $P < 0.01$. **: significant at the 0.01 ns: non-significant ans: almost non-significant.

Table 15. Correlation coefficients between SSa, SSd, Sum ∑a+d quantity and Slope.

Root concentration	Similarity matrix correlations				
	Correlation	SSa	SSd	Sumad	Slope
SSa	Pearson Correlation	1			
	Sig. (2-tailed)				
	N	20			
SSd	Pearson Correlation	0.629**	1		
	Sig. (2-tailed)	0.003			
	N	20	20		
Sumad	Pearson Correlation	0.828**	0.956**	1	
	Sig. (2-tailed)	0.000	0.000		
	N	20	20	20	
Slope	Pearson Correlation	-0.012	-0.074	-0.058	1
	Sig. (2-tailed)	0.958	0.756	0.808	
	N	20	20	20	20

**. Correlation is significant at the 0.01 level (2-tailed).

lites were characterized with relevant biological activity among which saikosaponins exhibited potent anti-inflammatory, hepatoprotective and immunomodulatory activities both *in vivo* and *in vitro* (Ashour and Wink, 2011); also antioxidant and antimicrobial properties (Shafaghat, 2011). The pharmacological experiments demonstrated that the SSd activity > SSa activity > SSc activity (Pan, 2006). The latter constatation linked to our work showed *B. smithii*, *B. sibiricum* and *B. scorzonerifolium* looked of great interest because of their high SSd roots content in which the –OH of C-23 is necessary for their pharmacological effects and the α-OH of C-16 pharmacological activity higher than the β-OH bioactivity. Future investigations and screenings could be sharpened on the possible bioactivity interaction of the saikosaponin d acting as antiviral agent in HIV research. Focusing on this debate, due to its pharmacological activity, future research oriented to SSd (particularly *B. smithii*, *B. sibiricum* and *B. scorzonerifolium*) could be led as the treatment of specific disease involves numerous plants or the development of different plant molecules. There is prospective potency for some of these species, therefore, it would be interesting to severalise them in their prevailing constituents and deepen the necessary research for a better knowledge.

Such programs will provide basis for pharmaceutical companies to improve generating capacity and increase profits.

Table 16. ANOVA of SSa, SSd, Sum ∑a+d quantity and Slope.

Root concentration	ANOVA					
	Correlation	**Sum of squares**	**df**	**Mean Square**	**F**	**Significance**
SSa	Between Groups	66197	12	5516	0.295	0.969^{ns}
	Within Groups	130892	7	18699		
	Total	197089	19			
SSd	Between Groups	366680	12	30557	0.598	0.793^{ns}
	Within Groups	357436	7	51062		
	Total	724116	19			
Sumad	Between Groups	586564	12	48880	0.423	0.909^{ns}
	Within Groups	809507	7	115644		
	Total	1396071	19			

ns: Correlation non-significant at the 0.01 level.

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Conflict of Interest

The authors have no conflict of interest.

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