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Saponin profile of wild asparagus species

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ABSTRACT

The aim of this work was to study the saponin profiles from spears of different wild asparagus species in the context of its genetic diversity aside from geographical seed origin. They included *Asparagus pseudoscaber* Grecescu, *A. maritimus* (L.) Mill., *A. brachiphyllus* Turcz., *A. prostrates* Dumort. and *A. officinalis* L. The saponin analysis by LC-MS has shown that saponin profile from wild asparagus is similar to that previously described for *triguero* asparagus from Huétor-Tájar landrace (*triguero* HT), which had not ever been reported in the edible part of asparagus. All the samples, except *A. officinalis*, were characterized for having saponins distinct to protodioscin and the total saponin contents were 10-fold higher than those described for commercial hybrids of green asparagus. In particular, *A. maritimus* from different origins were rich in saponins previously found in *triguero* HT. These findings supported previous suggestion, based on genetic analysis, about *A. maritimus* being the origin of *triguero* HT. Multivariate statistics including Principal Component Analysis and hierarchical clustering analysis were used to define both similarities and differences among samples. The results showed that the greatest variance of the tested wild asparagus could be attributed to differences in the concentration of particular saponins and this knowledge could be a tool for identifying similar species.

KEYWORDS: wild asparagus; saponins; HPLC-MS, principal component analysis; phytochemical profile

Introduction

The worldwide consumption of wild vegetables has played an important role in complementing staple agriculture foods (Morales and others 2012). Although the traditional use of non-cultivated vegetables has decreased with the development of agriculture and global supply chains, some species are still consumed. Indeed, an increasing interest in wild edible plants has been observed in modern societies, due to the fact that wild species are considered a great potential source of unusual flavors and because of their nutritional and pharmaceutical properties (Ertuğ 2004; Ogle and others 2004; Tardío and others 2006; Barucha and Pretty 2010; Sánchez-Mata and others 2011).

Among wild plants, the *Asparagus* genus has a relevant position, including over 250 species of both food and medicinal interest (Bozzini 1959). Although *A. officinalis* L. is the only cultivated asparagus nowadays, other wild species are edible and traditionally consumed. Wild asparagus is considered a healthy food and used in folk medicine as a diuretic to treat several kidney related disorders (Guarrera and Savo 2013). Nowadays, there is an increasing scientific interest in studying the health benefits of these wild asparagus because not only their nutritional properties, but also their richness in bioactive compounds with demonstrated health-promoting properties (García-Herrera and others 2014; Ferrara and others 2011) such as carotenoids, phenols, saponins, ascorbic acid and other organic acids, among others (Tardío and others 2016; Morales and others 2012; Sánchez-Mata and others 2011; Guillén and others 2008).

On the other hand, several studies have shown that the contents and the types of phenols and fatty acids are higher in wild asparagus than in cultivated one (Morales and others 2012; Guillén and others 2008). In the same way, it

has been observed that the carotenoid content is higher in wild asparagus and it has been reported significant differences among distinct species (García-Herrera and others 2014).

Saponin content may be affected by the season, the climate and altitude, the plant organ (shoots, roots, fruits, flowers) and the species of *Asparagus* genus (Negi and others 2011). Similarly, it is known that the saponin content is higher in white asparagus than in green one (Lee and others 2010). In 2000, the European Commission registered the *triguero* Huétor Tájar (*triguero* HT) asparagus as Protected Geographical Indication in the European Union (European Commission, 2000). The *triguero* HT asparagus, also known as "t*riguero*", is the only tetraploid asparagus cultivar, together with the Italian "Violetto d'Albenga" in Europe (Moreno and others 2006). Previous studies on strain identification and phylogeny have suggested that the *triguero* HT asparagus is close to *A. officinalis* and *A. maritimus* (Moreno and others 2008a,b). Our studies have revealed that among green asparagus, the total saponin content was much higher in *triguero* HT than in commercial hybrids (CH) (Vázquez-Castilla and others 2013a). However, the information about the saponin profile in wild asparagus is scarce (Negi and others 2011).

The most significant sources of saponins in human diet are triterpenoid types from the legumes such as soybeans and chick peas, typically described in cultivated crops. Steroids saponins are less common and can be found in foods such as asparagus and yucca. The structure of saponins provokes a characteristic behavior at hydrophobic–hydrophilic interfaces and subsequently on membrane cell permeabilisation which is critical for it activity (Sudji and others 2015). In fact, the consumption of saponins is associated to reduced plasma cholesterol concentrations (Harwood and others 1993) and then the risk

of coronary heart diseases. Other important activity described for saponins is the induction of cancer cell death through different pathways, including apoptosis (Lorent and others 2014). These important beneficial health activities, among others, support the interest of finding new sources of saponins in human diet.

In a previous work, we have developed a new LC-MS method, requiring fairly simple equipment and yielding a clean mixture of saponins that were easily resolved and quantified (Vázquez-Castilla and others 2013a). The results showed that the proposed method can be useful for the profiling of saponin in different asparagus genotypes and, therefore, for the differentiation of asparagus types (Vázquez-Castilla and others 2013a,b). Commercial hybrids and white asparagus are characterized as mostly having an unique steroidal saponin furostanol type, protodioscin, which is a glycoside derivative of diosgenin (Lee and others 2010; Vázquez-Castilla and others 2013a). However, when we have studied the flavonoids and saponin composition from different *triguero* HT germ plasm genotypes we have found that *triguero* HT asparagus has a distinct flavonoid and saponin profile compared to CH (Vázquez-Castilla and others 2013a,b; Fuentes-Alventosa and others 2008). Obtained results revealed that, while CH contains protodioscin and rutin as the major saponin and flavonoid respectively, *triguero* HT presents a more complex profile. This consists on a combination of protodioscin and at least twelve different new saponins derived from a furostanol-type steroidal genin with a single bond between C5 and C6 of the B ring, in addition to eight different flavonoids and a lack or very low content of rutin. It is interesting to point out that the morphological characteristics of *triguero* HT asparagus are similar to those found in wild asparagus: thinner and bitter spears, shorter cladodes, slightly and

striated stems. All these characteristics of the different *triguero* HT genotypes might be related to the fact, pointed out for some authors, that the HT landrace could be a hybrid between cultivated diploid varieties of *A*. *officinalis* and wild *A*. *maritimus* (Moreno and others 2008a).

The aim of this research was to study the saponin profile present in the wild asparagus spears from different species in order to find relationships between the wild species and *triguero* HT landrace.

Materials and methods

Plant material

The samples evaluated consisted on spears from 5 species of wild asparagus (*A. pseudoscaber* Grecescu*, A*. *maritimus* (L.) Mill.*, A*. *brachiphyllus* Turcz.*, A*. *prostratus* Dumort*. and A*. *officinalis L.*). Each seed samples were collected from different geographical localization. Samples of *A*. *officinalis* and *A*. *brachyphyllus* were from Russia, *A*. *pseudoscaber* from Czechia, *A*. *prostratus* from Spain (Galicia) and A. *maritimus seeds*, due to its abundance, were collected in different places, such as Italy (Padova and Venice), Albania and Spain (Cartagena). All the spears were harvested from experimental fields (Córdoba, Spain) and immediately transported to the laboratory where they were trimmed to a final length of 21 cm, weighed and frozen at -20 °C. All samples were harvested in the same experimental fields in order to eliminate the variables related to environmental and agronomic conditions.

Chemicals and reagents

142 Protodioscin (97%) and shatavarin (98.6%), with purity checked by NMR,
143 were purchased from Chromadex Chemical Co. (Barcelona, Spain). Ethanol,
144 formic acid (96%) and acetonitrile, high-performance liquid chromatography
145 (HPLC) grade, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).
146 Pure deionized water was obtained from a MiliQ50 system (Millipore
147 Corporation, Bedford, MA 01730 U.S.A.).

148 **Saponin extraction**

149 Samples, consisting of 25 g of fresh material, were extracted with 100 mL
150 of 80% ethanol in an Ultraturrax (T25) (IKA-Labortechnik, Staufen, Germany) for
151 1 minute at maximum speed and filtered. The residue was extracted again in
152 the same conditions. The ethanol extracts were pooled together and evaporated
153 to dryness at reduced pressure. All extractions were made in triplicate. The
154 dried ethanol extract was re-dissolved in 50 mL of EtOH 80% and 1 mL of this
155 dissolution was centrifuged at 12.000 rpm for 3 min and injected into the HPLC-
156 MS system.

157 **Saponin analysis by HPLC-MS**

158 The method for saponin analysis was developed by our research Group
159 and was described previously in detail (Vázquez-Castilla and others., 2013a).
160 Briefly, a HPLC Waters Alliance system fitted to a MEDITERRANEAN SEA18
161 reverse-phase analytical column (25 cm length x 4.6 mm id., 5 µm particle size;
162 Teknokroma, Barcelona) was used. An elution gradient was used with solvent A
163 (water with 1% formic acid) and B (acetonitrile with 1% formic acid): 0-30 min,
164 20% B; 30-60 min, linear gradient to 30% B; 60 to 70 min linear gradient to
165 100% B and 70-80 min, linear gradient 20% B.

The saponins were detected using an on-line connected quadrupole mass analyzer (ZMD4, Micromass, Waters, Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 V (negative mode) and 50 V (positive mode) with scans from *m/z* 200 to 1200. Capillary voltage was 3kV; the desolvation temperature was 200 ºC; source temperature 100 ºC and extractor voltage 12 V. The flow rate was kept at 1 mL/min and a split ratio of 5:1 for each analysis.

Quantitative analysis

The external standard method was used for the quantification of asparagus saponins (Vázquez-Castilla and others., 2013a). For each standard, the selected ion chromatogram corresponding to its molecular ion in negative mode at 100 V, was integrated and the peak area was plotted against concentration and subjected to regression analysis.

Statistical analysis

Results were expressed as mean value ± standard deviation. To assess for differences in the total content of saponins in the different species, a multiple sample comparison was performed using the Rcmdr package, R software v2.15.2. (Available at www.r-project). Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to contrast the groups. The level of significance was $p < 0.05$. Two matrices were prepared: one containing type of saponin variables, the other contained species-origin of the seed variables. These were subjected to the principal component analysis (PCA) and hierarchical clustering analysis (HCA) in order to display maximum variance in a data profile by finding a linear combination of the initial variables. Each component in the PCA model is characterized by two

attribute percentages of variance, describing the correlations of variables, and eigenvalues describing differences or similarities among the samples. The analysis was conducted using an Rcmdr package, R software v2.15.2. (Available at www.r-project.), the level of $P<0.05$ being considered significant.

Results

Saponin profile of wild asparagus

In this study, some of the most important wild asparagus species in Europe have been analyzed, as described in “Plant material” section. The identification of saponins was made by the method that we have previously developed (Vázquez-Castilla and others 2013a). This is based on assigning molecular ions tandem MS fragments by the application of a liquid chromatography−mass spectrometry (LC−MS). This methodology allowed the detection of several peaks, which were classified by their retention time, molecular weight and fragmentation pathway, and as well as the co-injection with authentic reference saponins previously purified and identified from *triguero* HT (Vázquez-Castilla and others 2013a,b). The fragmentation pathway has been studied through the mass spectra obtained in negative (100 V-) and positive (50V+) modes and showed that the pattern of saponins from wild species is similar to that described for different asparagus genotypes of *triguero* HT. However, *A. officinalis*, both from wild and cultivated spears, is characterized by protodioscin as unique saponin present likewise it has been described in CH (Table 1) (Vázquez-Castilla and others 2013a,b).

As it can be observed in Table 1, *A. prostratus* contained a mixture of protodioscin and HTSAP-4. The saponin profile of *A. maritimus* from different

geographical localization seed mainly consisted on saponins previously reported for *triguero* HT, which are accompanied by a minor saponin (WSAP-3). This compound presented the same molecular weight and pathway fragmentation model as that previously reported for the HTSAP-6 from *triguero* HT (Vázquez-Castilla and others 2013a,b), although it presented different retention time, (WSAP-3 Rt= 34.7 min *vs* HTSAP-6 Rt=25.03). In addition, those have been detected other three tentative saponins (WSAP-1, WSAP-2, and WSAP-4) with a molecular weights that have not been previously described.

The mass spectra of WSAP-1 (Figure 1) in negative and positive modes were compatible with a saponin containing two deoxyhexose and three hexoses. Figure 1A showed the product ions originated in negative mode from the molecular ion $[M-H]^-$ (m/z 1211) by loss of either a deoxyhexose m/z 1065 or a hexose m/z 1049. The ion m/z 903 was originated from the loss of a hexose from the ion m/z 1065 or a deoxyhexose from the ion at m/z 1049. The ion at m/z 757 was originated from the loss of a deoxyhexose, the ion at m/z 595 from the loss of a hexose and the ion at m/z 433 (deprotonated genin) from another hexose loss. In the case of the positive spectrum mode (Figure 1B), it showed the ions at m/z 1235 (sodium adduct m/z 1195 and loss of one H_2O molecule) and the ions at m/z 1049, m/z 887, m/z 725, m/z 579 and m/z 417 corresponding to the loss of a deoxyhexose, two hexoses and two deoxyhexoses, respectively.

The second peak, named WSAP-2, had a sugar fragmentation order similar to WSAP-1 (Figure 2). In the spectrum in negative mode (Figure 2A), ions could be seen at m/z 903, m/z 757, m/z 595 and m/z 433 (deprotonated genin) resulting from consecutive losses of a hexose, a deoxyhexose, a hexose and a hexose, respectively. Similarly, in the positive mode spectrum (Figure 2B)

242 ions appeared at m/z 1089 (sodium adduct molecule), m/z 1049 (loss of one
243 H_2O molecule) and ions at m/z 903, m/z 741, m/z 579 and m/z 417 resulting
244 from the loss of a deoxyhexose, a hexose, a hexose and a hexose,
245 respectively.

246 For *A. maritimus* from Cartagena (Spain) and Albania these new
247 tentative saponins represented approximately 15% of the total content (WSAP-4
248 and WSAP-1 respectively). However, it is remarkably that in *A. maritimus* from
249 Venice, *A. pseudoscaber* and *A. brachiphyllus* the main saponin was the new
250 saponin, named WSAP-4. This compound could be also a furostanol saponin as
251 shown by the prominent $[M+H-H_2O]+$ ion (m/z 861) in the positive mode. Unlike
252 the previously described saponins in the negative mode, no fragmentation ions
253 were detected (Figure 3A). In the positive mode (Figure 3B) there was some
254 fragmentation but only two sugars could be identified, so it was not possible to
255 provide a tentative composition for this novel saponin.

256

257 **Quantification and statistical analysis of saponins from wild asparagus**
258 **spears**

259 The saponins from eight lines of wild asparagus from different
260 geographical origin seeds were determined and quantified by the proposed LC-
261 MS method under the conditions previously described. The two available
262 commercial standards were protodioscin and shatavarin IV: the first is a
263 furostanoid saponin with a double bond between carbons 5 and 6 in the B-ring,
264 and the latter is a spirostanoid saponin with a single bond between carbons 5
265 and 6 of the B-ring (Figure 4). It is remarkable that in the method that we have
266 used they have the same response factor (Vázquez-Castilla and others 2013a).

Since the new saponins are structurally related to both standards, the quantitative data provided in this study can be considered a good approximation to the real values and, in any case, useful for the purpose of comparison between different wild and cultivated species.

The results showed that there were significant differences in the composition of saponins from the investigated wild asparagus spears (Table 1). The total saponin content of the five species of wild asparagus studied are higher than those described in the CH and *triguero* HT (Lee and others 2010; Vázquez-Castilla and others 2013b; Wang and others 2003). Similarly, it has been described that the content for other phytochemicals, such as carotenoids, were higher in wild asparagus than in CH (García-Herrera and others 2014). The highest values of total saponins were found for *A. maritimus* from Cartagena (Spain) and *A. brachiphyllus* (143 and 224 mg/100 g FW, respectively). Remarkably, the highest saponin value of *A. maritimus* samples from Cartagena (Spain), revealed that the saponin content was affected not only for species but also by the seed origin.

Finally, in order to get a deep knowledge about the different composition of the saponin profile, we subjected the results to PCA and HCA analyses. A graphical presentation of the PCA results for the two sets of data in the form of biplots is shown in Figure 5. The types of saponins are displayed as vectors. In PC1 we can see that there is a negative relationship between the presence of WSAP-1, WSAP-2 and HTSAP-12 versus HTSAP-6 and HTSAP-7. In PC2, positive values were associated with the presence of WSAP-3, HTSAP-3 and HTSAP-8, while negative values with the presence of WSAP-4 and HTSAP-1, (Figure 5). In this particular regression analysis, the first two principal

292 components explained 96% of the independent saponin composition of the
293 variance in the qualitative data set.

294 The data were subjected to HCA analysis to assess the heterogeneity
295 among different species (Figure 6). As compared to PCA, HCA allows an
296 interpretation of the results in a fairly intuitive graphical way. The cluster
297 analysis of the different wild asparagus samples, according to saponin profile,
298 showed two clear clusters (cluster 1 and 2). Cluster 1 gathered all samples
299 analyzed but *A. maritimus* from Cartagena (Spain) that constituted cluster 2 with
300 a very different composition. Respect to cluster 1 is constituted for two main
301 groups characterized by the content in HTSAP-8. Cluster 1a referred to species
302 with low content and was constituted for the five species while cluster 1b is
303 comprised only by *A. maritimus* from Albania. Inspection of cluster 1a showed
304 that the content in WSAP-4 classified the samples in other two cluster: the *A.*
305 *maritimus* from Padova (Italy) and Venice (Italy) (cluster 1aa) and the rest of
306 samples (*A. brachiphyllus, A. pseudoscaber A. officinalis* and *A. prostratus*)
307 were clustered together (cluster 1ab). Within cluster 1ab, differences in saponin
308 composition are apparently dominant over species. *A. prostratus* samples were
309 found to be the most closely related to *A. officinalis* being protodioscin also
310 found as the major saponin (70%), as showed in Table 1. However, as it will be
311 discussed below, in the rest of the wild asparagus studied, other saponins were
312 predominant and distant from *A. maritimus* from Albanian samples (cluster 1b).

313

314 **Discussion**

315 It has been widely reported in bibliography that the saponin composition
316 is different between wild and cultivated species such as potatoes (Savarese and

317 others 2009), leeks (Zolfaghari and others 2006), soybean seeds (Tsukamoto
318 and others 1993) and legumes (Sotelo and others 1995). In the case of the
319 *Asparagus* genus, important differences were also found. Protodioscin is the
320 main saponin described in the spears of *A. officinalis* (Lee and others 2010;
321 Sharma and others 2009) the only cultivated asparagus species nowadays,
322 while in the wild spears of *A. racemosus,* shatavarin has been found as the
323 main saponin (Sharma and others 2009). We have previously reported that in
324 the spears from *triguero* HT, saponins were derived from a furostanol genin
325 different from protodioscin (Vázquez-Castilla and others 2013b). In the present
326 study, we have extended the analysis to several species of wild asparagus and
327 the comparison of results with data from our previous studies (Vázquez-Castilla
328 and others 2013a,b), has revealed that wild asparagus, except *A. officinalis*,
329 contained mixtures of saponins such as described in *triguero* HT and some
330 minority saponins unknown so far. Besides, based on the ESI mass spectra it
331 could be elucidate that these detected saponins are derived from a furostanol
332 genin with a single bond between C5-C6 in the B-ring and two deoxyhexoses
333 and two hexoses attached. In all cases, these saponins were derived from a
334 furostanol genin which was different from diosgenin, just like the saponins
335 described in *triguero* HT (Vázquez-Castilla and others 2013b). The results
336 showed the similarity between wild species and *triguero* HT asparagus in
337 qualitative composition and the differences of both of them respect to CH.
338 Remarkably, WSAP-4 is the main saponin detected in *A. pseudoscaber*
339 (Czechia), *A. maritimus* (Venice) and *A. brachiphyllus* from Russia. Further
340 studies on WSAP-4 could be interesting in order to deduce the structure of this
341 saponin and found the possible relationship between organoleptic and
342 functional role in the different asparagus species. In the present work, we have
343 also found that *A. officinalis* is characterized for containing mostly protodioscin,

344 independently if they are cultivated or wild spears. The composition reported for
345 *A. prostratus* could be due to the fact that *A. prostratus* is a subspecies from *A.*
346 *officinalis* as some authors pointed out (Kay and others 2001).

347 On the other hand, the quantification of saponins from the samples
348 analysed revealed that they contain very high amounts. In fact, as far as we
349 know, these range values have no ever been described in the literature about
350 spears of green asparagus. It is interesting to point out that the *triguero* HT
351 presents similar saponins derived from a genin type furostanol but their
352 concentrations were between 10 to 100 times lower than those from wild
353 samples (Vázquez-Castilla and others 2013a,b).

354 These data support the fact that the qualitative and quantitative saponin
355 compositions of plant foods are influenced by cultivar, environmental factors,
356 and plant organ. The saponin profile of all the samples analyzed was subjected
357 to PCA and HCA to determinate the influence of the different factors above
358 cited on the studied phytochemicals. Our results showed that wild sample of *A.*
359 *officinalis* spears are constituted only by protodioscin, as described from CH
360 (Lee and others 2010; Vázquez-Castilla and others 2013b; Negi and others
361 2011) and *A. prostratus* is very similar to *A. officinalis* respect to saponin
362 content. Remarkably, the results provide evidence that *A. maritimus* from
363 Cartagena (Spain), could be possibly new specie that it deserves to be further
364 investigated. In fact, these results are in accordance with those described for
365 Spanish population of *A. maritimus* by others authors (Pedrol and other 2013).
366 They have recently considered as a different species and named as *A.*
367 *macrorrhizus* Pedrol, Regalado et López-Encina. Possibly, the species used in
368 the present research could be *A. macrorrhizus* Pedrol and not *A. maritimus.* In
369 addition to the different flavonoid profile previously described for this new

species (Regalado and others 2016), we have shown it has a distinct and characteristic saponin profile too. These could better explain the differences in saponin content found among the Spanish populations of *A. maritimus* investigated in this research.

Conclusions

In summary, this study has shown important differences in the saponin content of different samples. These differences have been found among the distinct species, especially between *A. officinalis* and the other species. The other major goal of the current study was to investigate these saponin compositions in the context of its genetic diversity aside from geographical seed origin. The results allow us to support the possible genetic origin of *triguero* HT, which suggested that these spears could be a hybrid between cultivated diploid varieties of *A. officinalis* and wild *A. maritimus* (Moreno and others 2008a). These differences could justify the functional properties which have been attributed to these asparagus from ancient times. So further studies are of interest to determine the possible structure-activity relationship. Results from several works that we have developed during the last decade related to phytochemical composition as new criteria of selection suggest that HT landraces constitute a valuable genetic resource that could help to enlarge the genetic background of modern cultivars. They could be used for the development of new varieties with improved organoleptic, functional, and nutritional characteristics in order to satisfice the worldwide inclination for the consumption of quality natural compounds from plant materials. Moreover, this research could assist in wild asparagus identification from its closely allied species.

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Author's contribution

Sara Jaramillo-Carmona collected test data, interpreted the results and drafted the manuscript. Rocío Rodríguez-Arcos and Ana Jiménez-Araujo helped to draft the manuscript. Sergio López performed the statistical analyses. Juan Gil and Roberto Moreno provided the samples and made the taxonomic classification. Rafael Guillén-Bejarano designed the study and interpreted the results.

Abbreviations used

ESI, electrospray ionization; FW, fresh weight; HCA, hierarchical clustering analysis; HT, Huétor-Tájar saponin; HTSAP, Huétor-Tájar saponin, PCA, principal component analysis; PD, protodioscin; Rt; retention time; WSAP, wild saponin

References

Barucha Z, Pretty J. 2010. The roles and values of wild foods in agriculture systems. Phil Trans R Soc B 365: 2913-2926.

Bozzini A. 1959. Revisione cito-sistematica del genere asparagus L. I: Le specie di asparagus della flora italiana e chiave per la loro determinazione. Cardiologia 12:199-264.

Ertuğ,F. 2004. Wild edible plants of the Bodrum Area (Mugla, Turkey). Turk J Bot 28:161–174.

European Commission 2000. Commission Regulation (EC) No 547/2000 of 14 March 2000 supplementing the Annex to Regulation (EC) No 2400/96 on the entry of certain names in the “Register of protected designations of origin and protected geographical indications” provided for in Council Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. Official Journal of the European Communities L67/43:8–11.

Ferrara L, Dosi R, Di Maro A, Guida V, Cefarelli G, Pacifico S, Mastellone C, Fiorentin, A, Rosati A, Parente A. 2011. Nutritional values, metabolic profile and radical scavenging capacities of wild asparagus (*A. acutifolius* L.*).* J Food Compos Anal 24:326-333.

Fuentes-Alventosa J, Jaramillo S, Rodríguez-Gutiérrez G, Cermeño P, Espejo JA, Jiménez-Araujo A, Guillén-Bejarano R, Fernández-Bolaños J, Rodríguez-Arcos R. 2008. Flavonoid profile of green asparagus genotypes. J Agric Food Chem 56:6977-6984.

García–Herrera P. Cortés M, Cámara C, Tardío J, Olmedilla-Alonso B. 2014. Carotenoid content of wild edible young shoots traditionally consumed in Spain (*Asparagus acutifolius* L., *Humulus lupulus* L., *Bryonia dioica* Jacq. and *Tamus communis* L.). J Sci Food Agric 94:1914-1916.

443 Guarrera PM, Savo V. 2013. Perceived health properties of wild and cultivated
444 food plants in local and popular traditions of Italy: A review. J Ethnopharmacol
445 146:659 -680.

446 Guillén R, Rodríguez R, Jaramillo S, Rodríguez G, Espejo JA, Fernández-
447 Bolaños J, Heredia A. 2008. Antioxidants from asparagus spears: Phenolics.
448 Acta Hort 776:246-254.

449 Harwood HJJr, Chandler CE, Pellarin LD, Bangerter FW, Wilkins RW, Long CA,
450 Cosgrove PG, Malinow MR, Marzetta CA, Pettini JL, Savoy YE, Mainet JT.
451 1993. Pharmacologic consequences of cholesterol absorption inhibition:
452 alteration in cholesterol metabolism and reduction in plasma cholesterol
453 concentration induced by the synthetic saponin beta-tigogenin cellobioside (CP-
454 88818; tiqueside). J Lipid Res 34:377-95.

455 Kay QON, Davies EW, Rich TCG. 2001. Taxonomy of the western European
456 endemic *Asparagus prostratus* (*A. officinalis* subsp. *prostratus*)
457 (Asparagaceae). Bot J Linn Soc 137*:*127-137.

458 Lee W, Yoo K, Patil B. 2010. Development of a rapid HPLC-UV method for
459 simultaneous quantification of protodioscin and rutin in white and green
460 asparagus spears. J Food Science 75:703-709.

461 Lorent JH, Quetin-Leclercq J, Mingeot-Leclercq MP. 2014. The amphiphilic
462 nature of saponins and their effects on artificial and biological membranes and
463 potential consequences for red blood and cancer cells. Org Biomol Chem.
464 12:8803-22

465 Morales P, Ferreira I, Carvalho A, Cortés M, Cámara M, Tardío J. 2012. Fatty
466 acids profiles of some Spanish wild vegetables. Food Sci Technol Int 18:281-90.

Moreno R, Espejo JA, Cabrera A, Millan T, Gil J. 2006. Ploidic and molecular analysis of “Morado de Huétor” asparagus (Asparagus officinalis L.) population: A Spanish tetraploid landrace. Genet Resour Crop Evol 53:729–736.

Moreno R, Espejo JA, Cabrera A, Gil J. 2008a. Origin of tetraploid cultivated asparagus landraces inferred from nuclear ribosomal DNA internal transcribed spacers’ polymorphisms. Ann Appl Biol 153:233-241.

Moreno R, Espejo JA, Moreno MT, Gil J. 2008b. Collection and conservation of “Morado de Huétor” Spanish tetraploid asparagus landrace. Genet Resour Crop Evol 55:773–777.

Negi J, Singh P, Pant G, Rawat M. 2011. High performance liquid chromatographic analysis of derivatized sapogenin of asparagus (RP-HOLC analysis of derivatized sapogenin of Asparagus). J Med Plant Res 5:1900-1904.

Ogle BM, Dung NNX, Do TT, Hambraeus L. 2004. The contribution of wild vegetables to micronutrient intakes among women. An example from the Mekong Delta, Vietnam. Ecol Food Nutr 40:159–184.

Pedrol J, Regalado JJ, López-Encina C. 2013. *Asparagus* L.:*Asparagus macrorrhizus* (Pedrol, Regalado & López-Encina), sp. nov., In: Rico E, Crespo MB, Quintanar A, Herrero A, Aedo C (eds) Flora Ibérica, vol XX. Real Jardín Botánico, CSIC, Madrid, 117–119.

Regalado JJ, Moreno R, Castro P, E. Carmona-Martín E, R. Rodríguez R, Pedrol, Larrañaga N, Guillén R, J Gil J, Encina CL. 2016. *Asparagus macrorrhizus* Pedrol, Regalado et López-Encina, an endemic species from Spain in extreme extinction risk, is a valuable genetic resource for asparagus breeding. Genet Resour Crop Evol 1-14 DOI: 10.1007/s10722-016-0456-2.

491 Sánchez-Mata MC, Cabrera RD, Morales P, Fernández-Ruiz V, Cámara M,
492 Díez C, Pardo-de-Santaya M, Tardío J. 2011. Wild vegetables of the
493 Mediterranean area as valuable sources of bioactive compounds. Genet Resour
494 Crop Evol 59:431-433.

495 Savarese S, Andolfi A, Cimmino A, Carputo D, Frusciante L, Evidente A. 2009.
496 Glycoalkaloids as biomarkers for recognition of cultivated, wild and somatic
497 hybrids of potato. Chem Biodivers 6:437-446.

498 Sharma U, Saini R, Bobita Y, Kuma RN, Singh, B. 2009. Steroidal saponins
499 from Asparagus *racemosus*. Chem Pharm Bull 57:890-893.

500 Sotelo A, Contreras E, Flores S. 1995. Nutritional value and content of
501 antinutritional compounds and toxics in ten wild legumes of Yucatan peninsula.
502 Plant Food Hum Nutr 74:115-123.

503 Sudji IR, Subburaj Y, Frenkel N, García-Sáez AJ, Wink M. 2015. Membrane
504 Disintegration Caused by the Steroid Saponin Digitonin Is Related to the
505 Presence of Cholesterol. Molecules 20:20146-20160

506 Tardío J, Sánchez-Mata MC, Morales R, Molina M, García-Herrera P, Morales
507 P, Díez-Marqués C, Fernández-Ruiz V, Cámara M, Pardo-de-Santayana M,
508 Matallana-González MC, Ruiz-Rodríguez BM, Sánchez-Mata D, Torija-Isasa
509 ME, Guil-Guerrero JL, Boussalah N. 2016. Ethnobotanical and Food
510 Composition Monographs of Selected Mediterranean Wild Edible Plants. In:
511 Sánchez-Mata MC, Tardío J (eds) Mediterranean Wild Edible Plants.
512 Ethnobotany and Food Composition Tables. Springer, New York, 273-470.

513 Tardío J, Pardo-de-Santayana M, Morales R. 2006. Ethnobotanical review of
514 wild edible plants in Spain. Bot J Linn Soc 152:27-71.

515 Tsukamoto C, Kikuchi A, Harada K, Kitamura K, Okubo K. 1993. Genetic and
516 chemical polymorphisms of saponins in soybean seed. Phytochem 34:1351-
517 1356.

518 Vázquez-Castilla S, Jaramillo-Carmona S, Fuentes-Alventosa JM, Jiménez-
519 Araujo A, Rodríguez-Arcos R, Cermeño-Sacristán P, Espejo-Calvo JA, Guillén–
520 Bejarano R. 2013a. Optimization of a method for the profiling and quantification
521 of saponins in different asparagus genotypes. J Agric Food Chem 61:6250-
522 6258.

523 Vázquez-Castilla S, Jaramillo-Carmona S, Fuentes-Alventosa JM, Jiménez-
524 Araujo A, Rodríguez-Arcos R, Cermeño-Sacristán P, Espejo-Calvo JA, Guillén–
525 Bejarano R. 2013b. Saponin profile of green asparagus genotypes. J Agric
526 Food Chem 61:11096-11108.

527 Wang M, Tadmor Y, Wu QL, Chin CK, Garrison SA, Simon JE. 2003.
528 Quantification of protodioscin and rutin in asparagus shoots by LC/MS and
529 HPLC methods. J Agric Food Chem 51:6132-6136.

530 Zolfaghari B, Barile E, Capasso R, Izzo AA, Sajjadi E, Lanzotti V. 2006. The
531 sapogenin atroviolacegenin and its diglycoside atroviolaceoside from *Allium*
532 *atroviolaceum.* J Natural Product 69:191-195.

TABLES:

Table 1. Saponin composition and total content in different wild asparagus species.

	% Saponins												Total content
Asparagus species (Seed origin)	HTSAP-1	WSAP-1	HTSAP-3	HTSAP-4	PD	WSAP-2	HTSAP-12	HTSAP-6	HTSAP-7	HTSAP-8	WSAP-3	WSAP-4	mg/100 g
A. officinalis (Russia)	N.D	N.D	N.D	N.D	100a	N.D	N.D	N.D	N.D	N.D	N.D	N.D	2.2 ± 0.02[a]
A. pseudoscaber (Czechia)	0.7 ± 0.02[a]	N.D	N.D	N.D	N.D	1.0 ± 0.06[a]	N.D	1.7 ± 0.1[a]	N.D	N.D	N.D	97 ± 0.9[a]	88.3 ± 6.6[b]
A. maritimus (Italy-Venice)	N.D	N.D	1.5 ± 0.08[a]	N.D	N.D	N.D	N.D	11.5 ± 0.7[b]	2 ± 0.1[a]	N.D	N.D	85 ± 2.6[a]	32.1 ± 3.4[b]
A. maritimus (Italy-Padova)	N.D	N.D	2.2 ± 0.1[a]	N.D	N.D	N.D	N.D	77.2 ± 8.0[c]	1.3 ± 0.3[a]	1.3 ± 0.1[a]	3.3 ± 0.3[a]	14.7 ± 1.1[b]	22.4 ± 2.0[b]
A. maritimus (Albania)	N.D	N.D	13 ± 1.0[b]	N.D	N.D	N.D	11.5 ± 1.2[a]	N.D	N.D	68 ± 1.4[b]	7.8 ± 1.1[b]	N.D	16.8 ± 1.2[c]
A. maritimus (Spain-Cartagena)	N.D	16.6 ± 1.3	N.D	N.D	N.D	1.6 ± 0.09[a]	74.3 ± 3.1[b]	N.D	N.D	4.5 ± 0.2[c]	N.D	3.1 ± 0.3[c]	142.7 ± 13[b]
A. brachiphyllus (Russia)	1 ± 0.04[a]	N.D	N.D	N.D	N.D	N.D	N.D	2 ± 0.2a	N.D	N.D	N.D	96 ± 3.0[a]	224.2 ± 22.0[d]
A. prostratus (Spain-Galicia)	N.D	N.D	N.D	25.4 ± 1.7	74.6 ± 4.2[b]	N.D	N.D	N.D	N.D	N.D	N.D	N.D	51.4 ± 4.1[b]

In each column, values with different letters are significantly different (P<0.05). N.D. Not detected; HTSAP, Huétor-Tájar saponin; WSAP, wild saponin; PD protodioscin

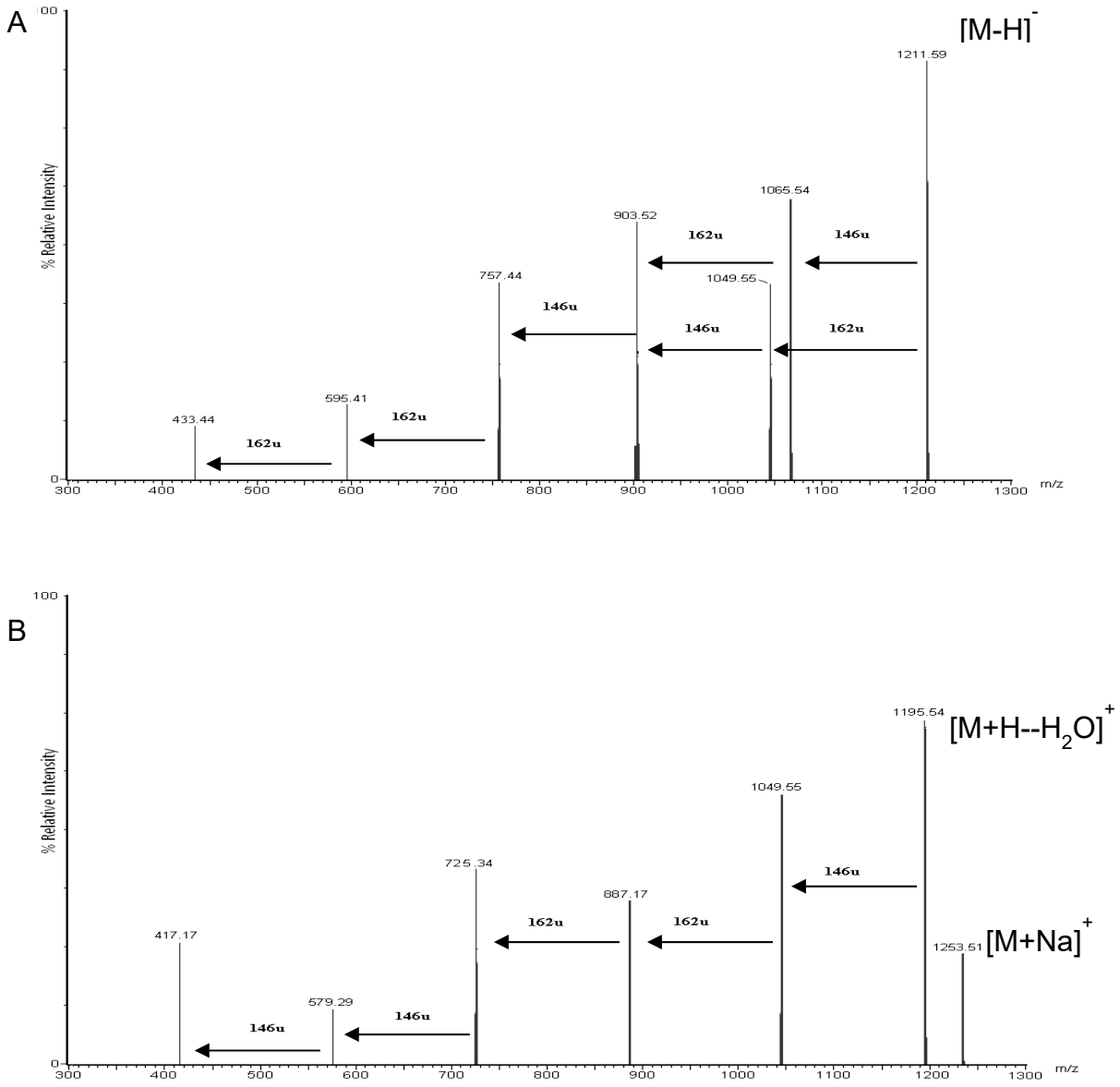


Figure 1 ESI mass spectra of WSAP-1 in negative (A, 100V-) and positive (B, 50V+) modes. Arrows indicate the loss of single monosaccharide moieties.

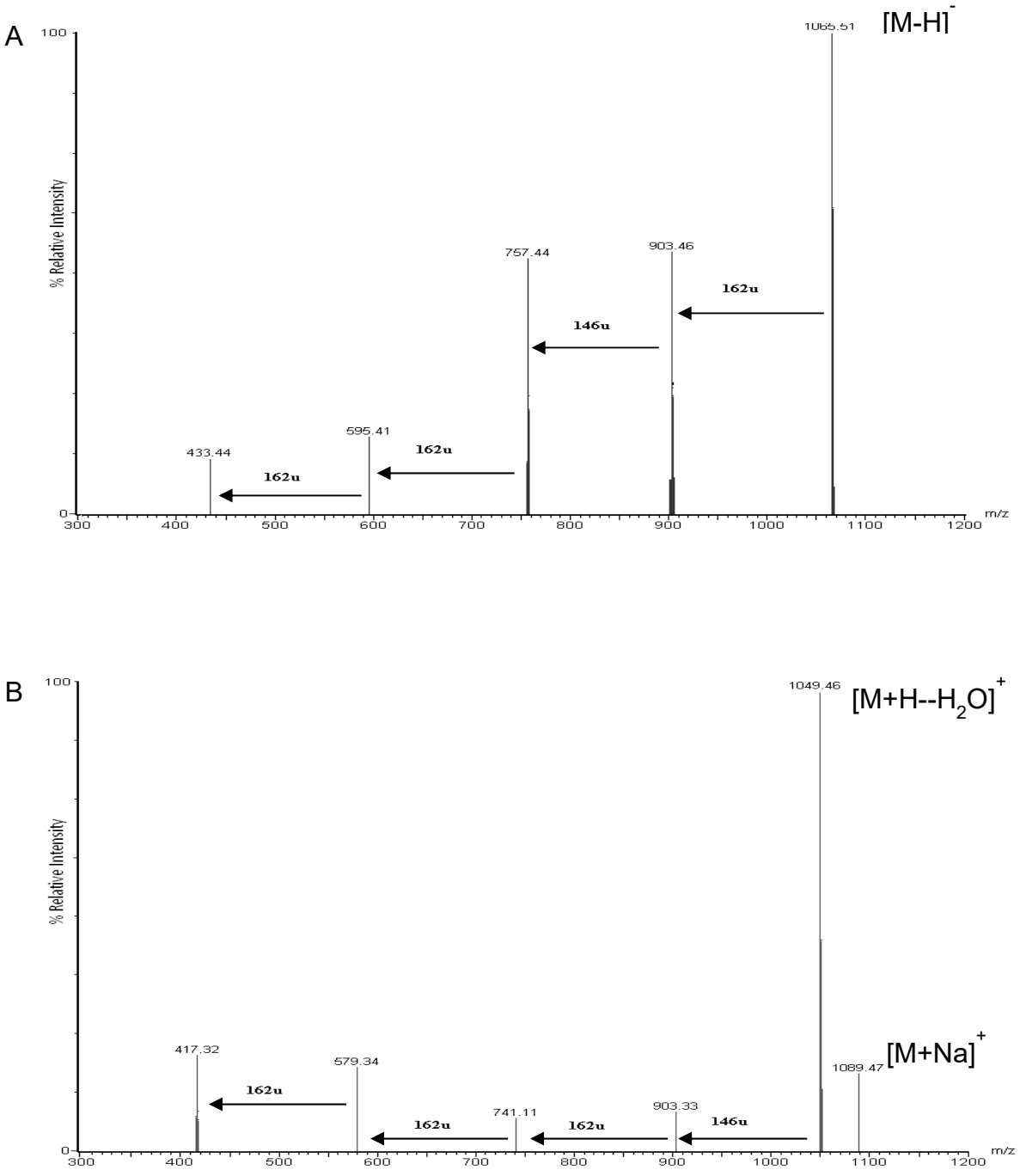


Figure 2 ESI mass spectra of WSAP-2 in negative (A, 100V-) and positive (B, 50V+) modes. Arrows indicate the loss of single monosaccharide moieties.

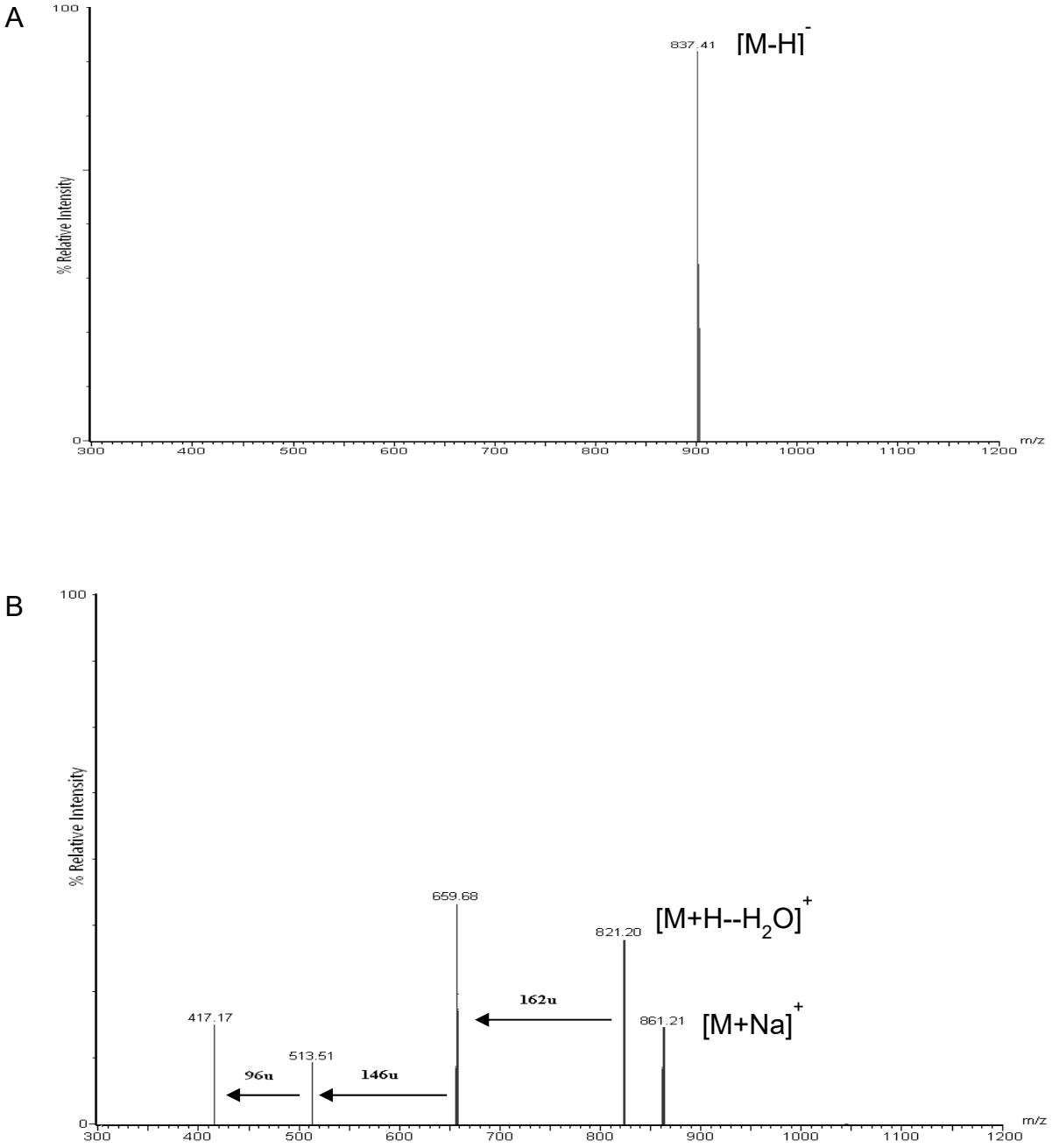


Figure 3 ESI mass spectra of WSAP-4 in negative (A, 100V-) and positive (B, 50V+) modes. Arrows indicate the loss of single monosaccharide moieties.

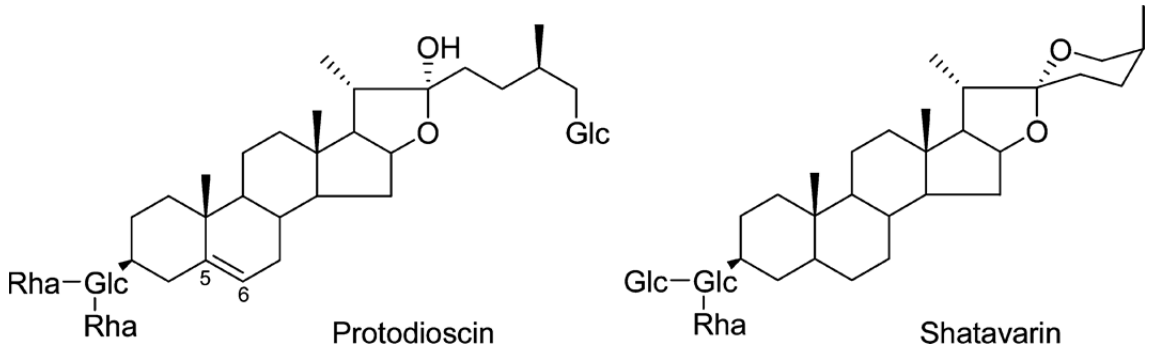


Figure 4 Structures of protodioscin and shatavarin.

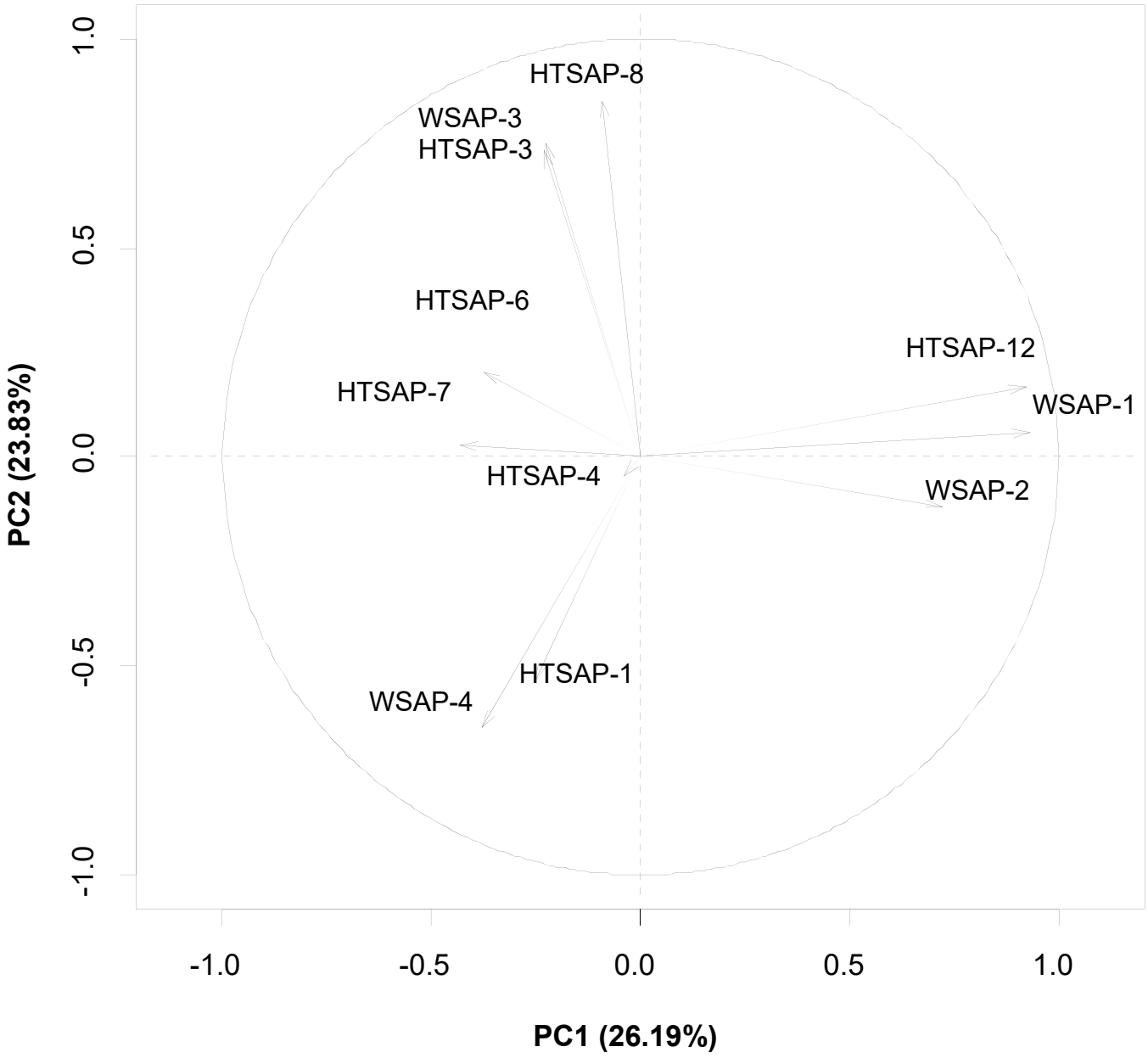


Figure 5 Principal component analyses of saponin profiles of different wild asparagus samples from different seed origin (n=35).

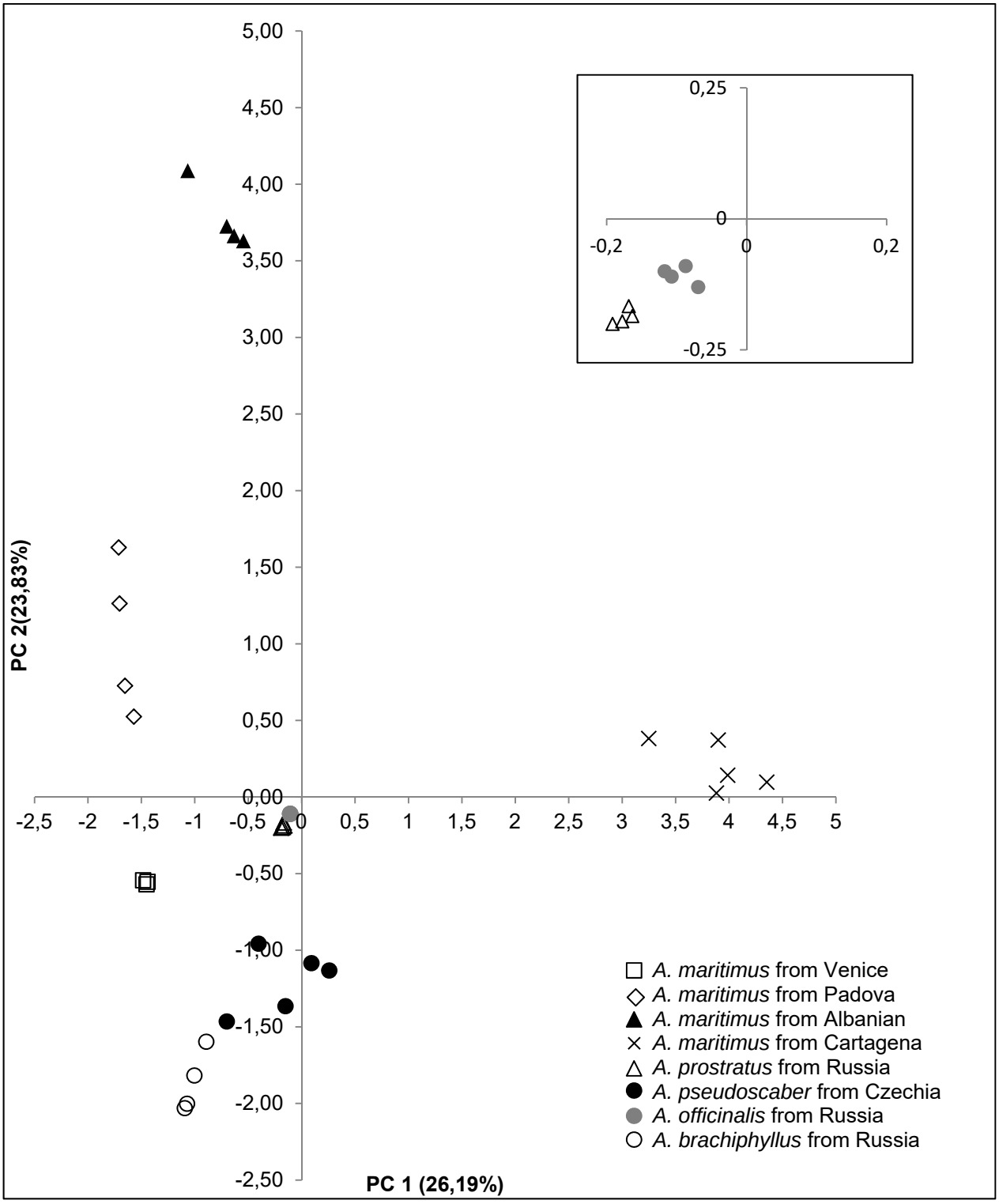


Figure 6 Hierarchical clustering analysis. The wild asparagus species clusters are located at the distinct positions described by two vectors of principal component 1 (PC1=26%) and principal component 2 (PC2=24 %). Inner Figure: data-zoom-image.

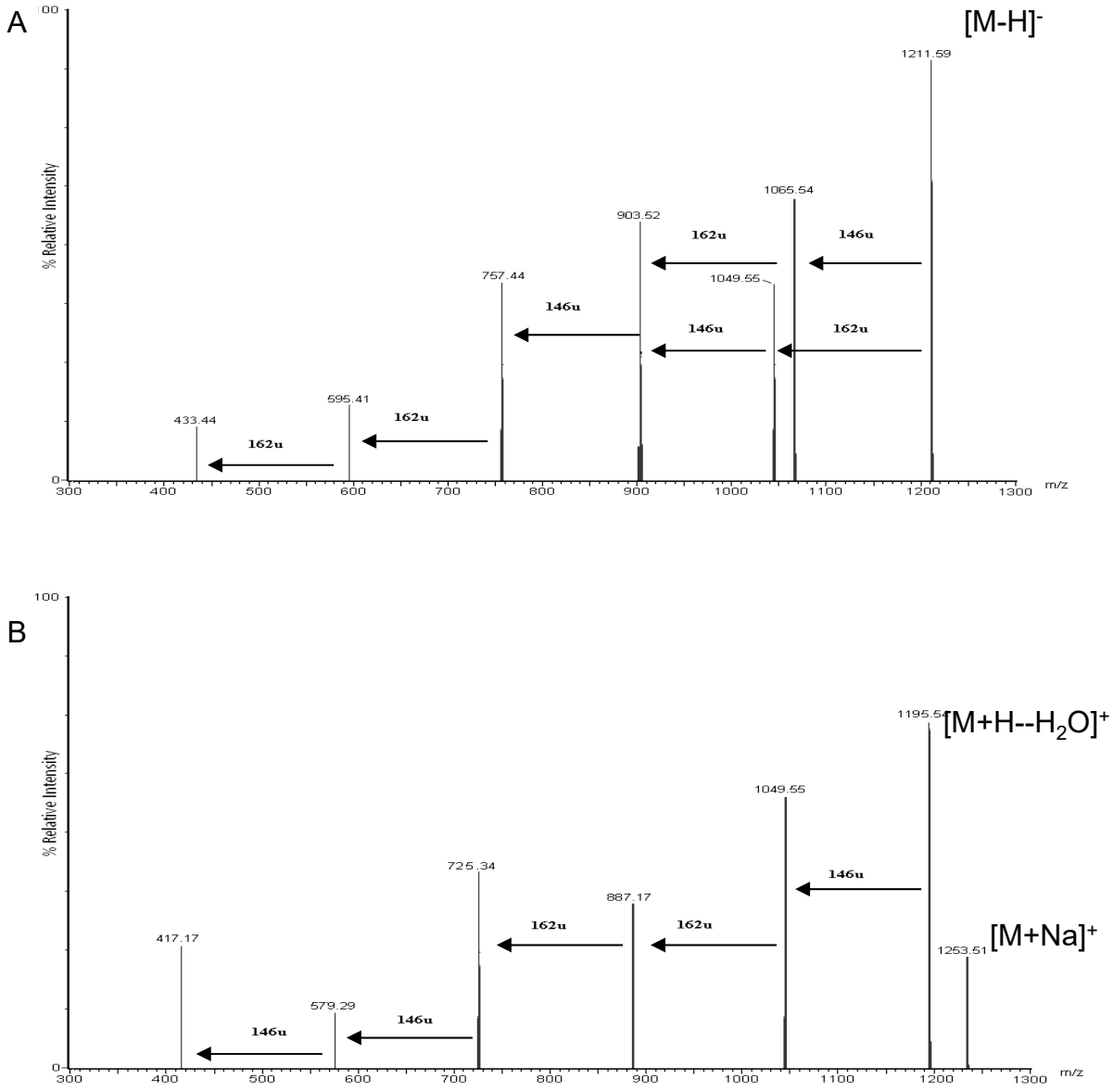


Figure 1 ESI mass spectra of WSAP-1 in negative (A, 100V-) and positive (B, 50V+) modes. Arrows indicate the loss of single monosaccharide moieties.

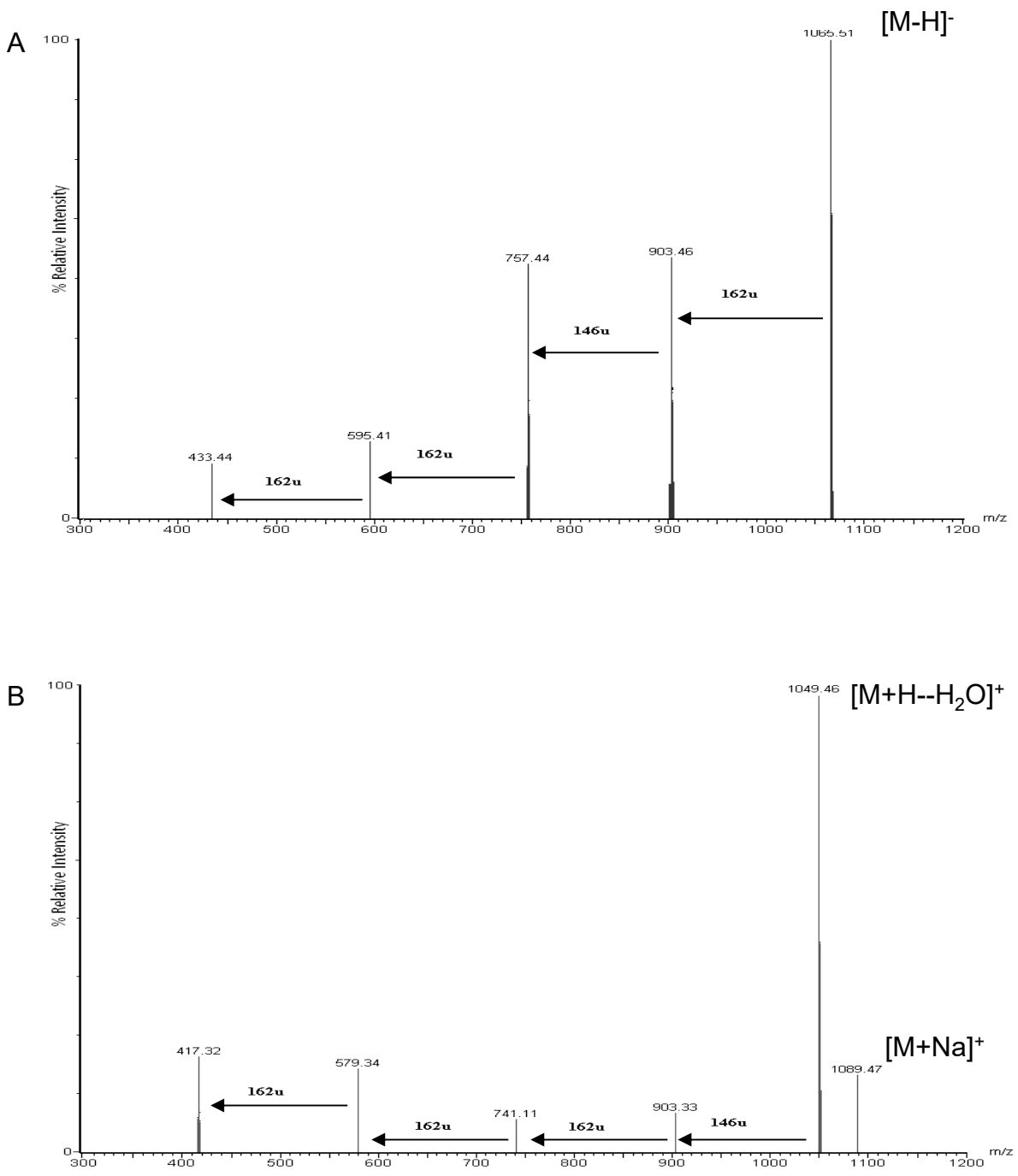


Figure 2 ESI mass spectra of WSAP-2 in negative (A, 100V-) and positive (B, 50V+) modes. Arrows indicate the loss of single monosaccharide moieties.

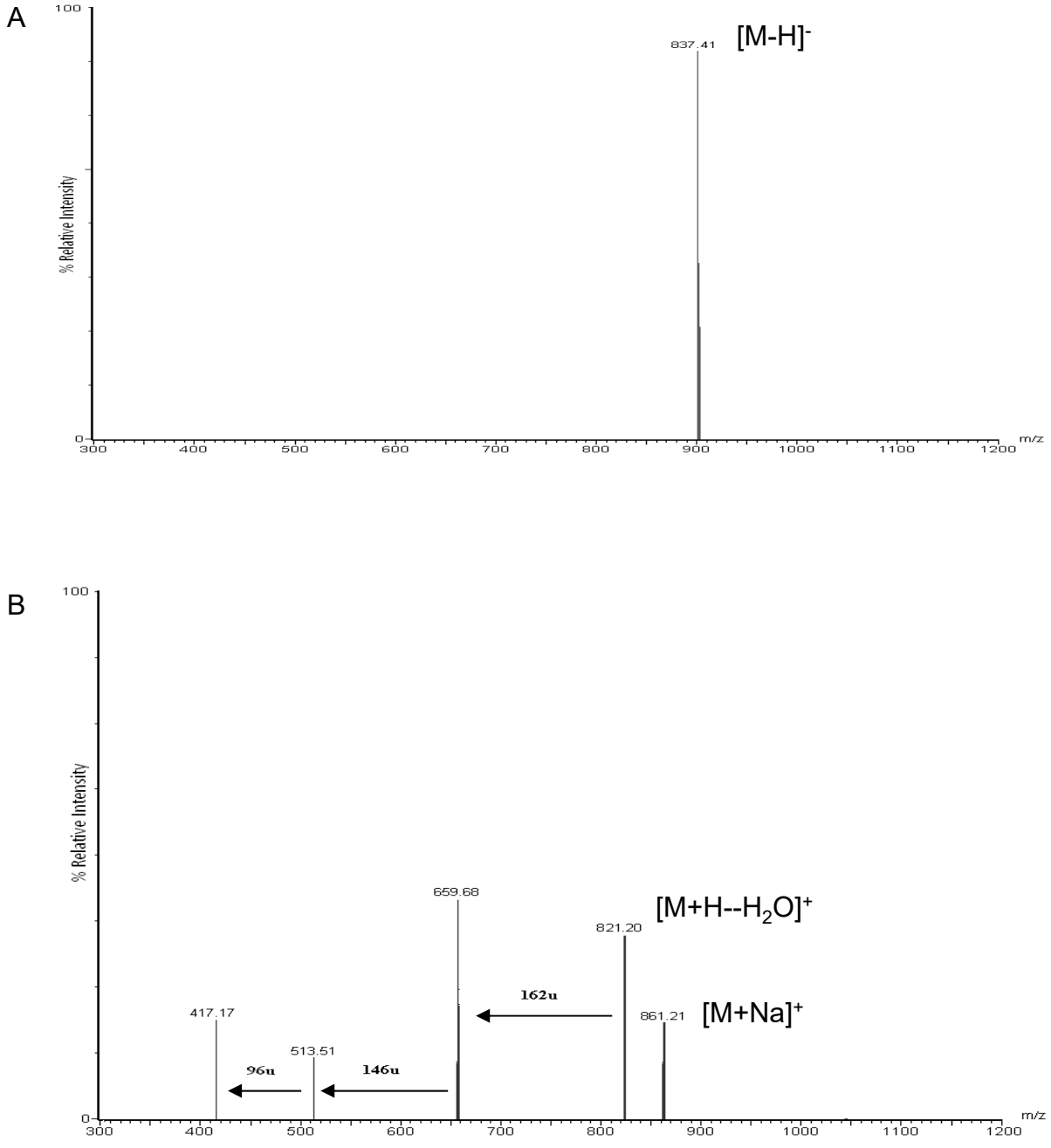
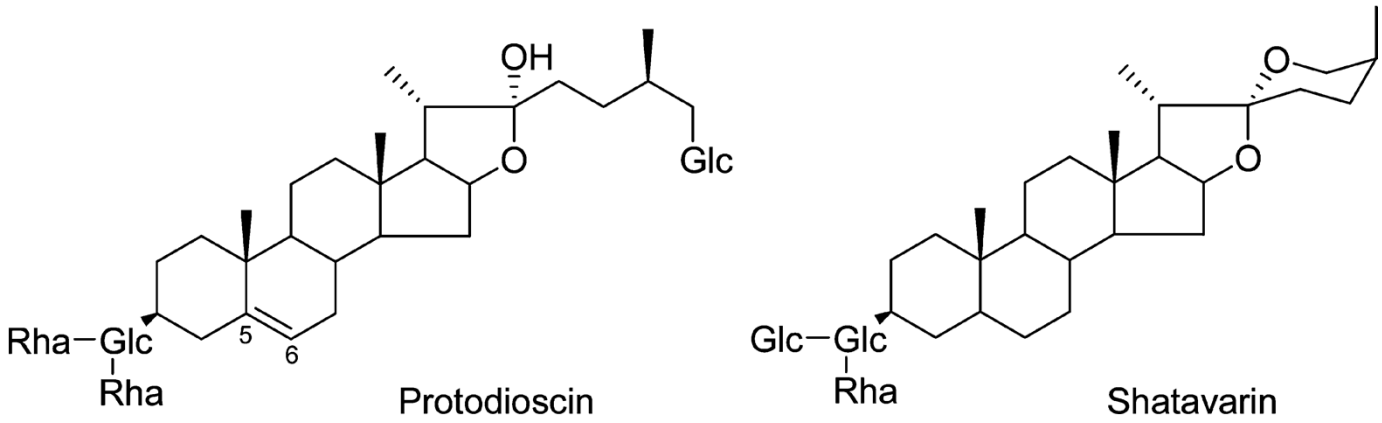


Figure 3 ESI mass spectra of WSAP-4 in negative (A, 100V-) and positive (B, 50V+) modes. Arrows indicate the loss of single monosaccharide moieties.



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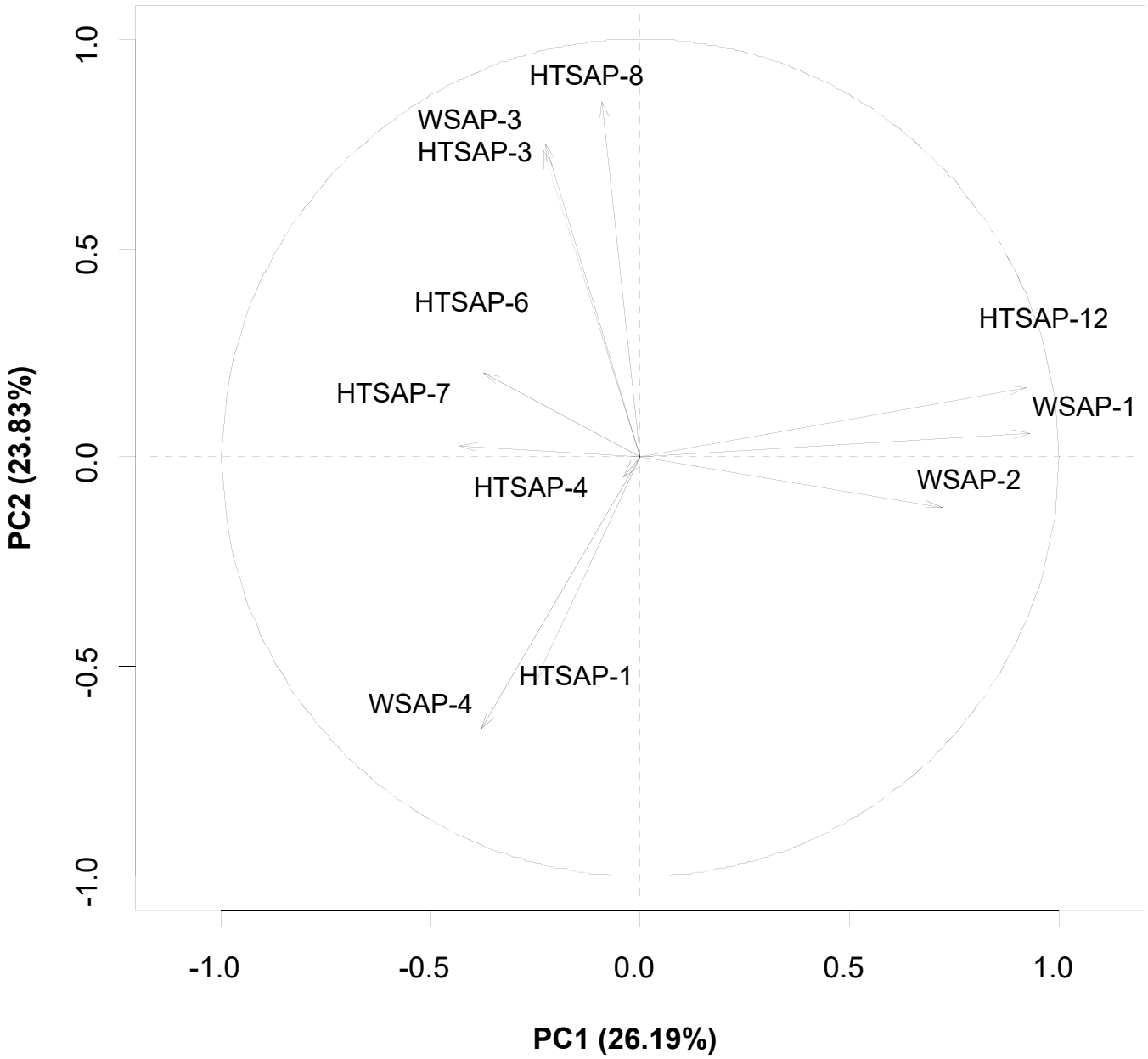
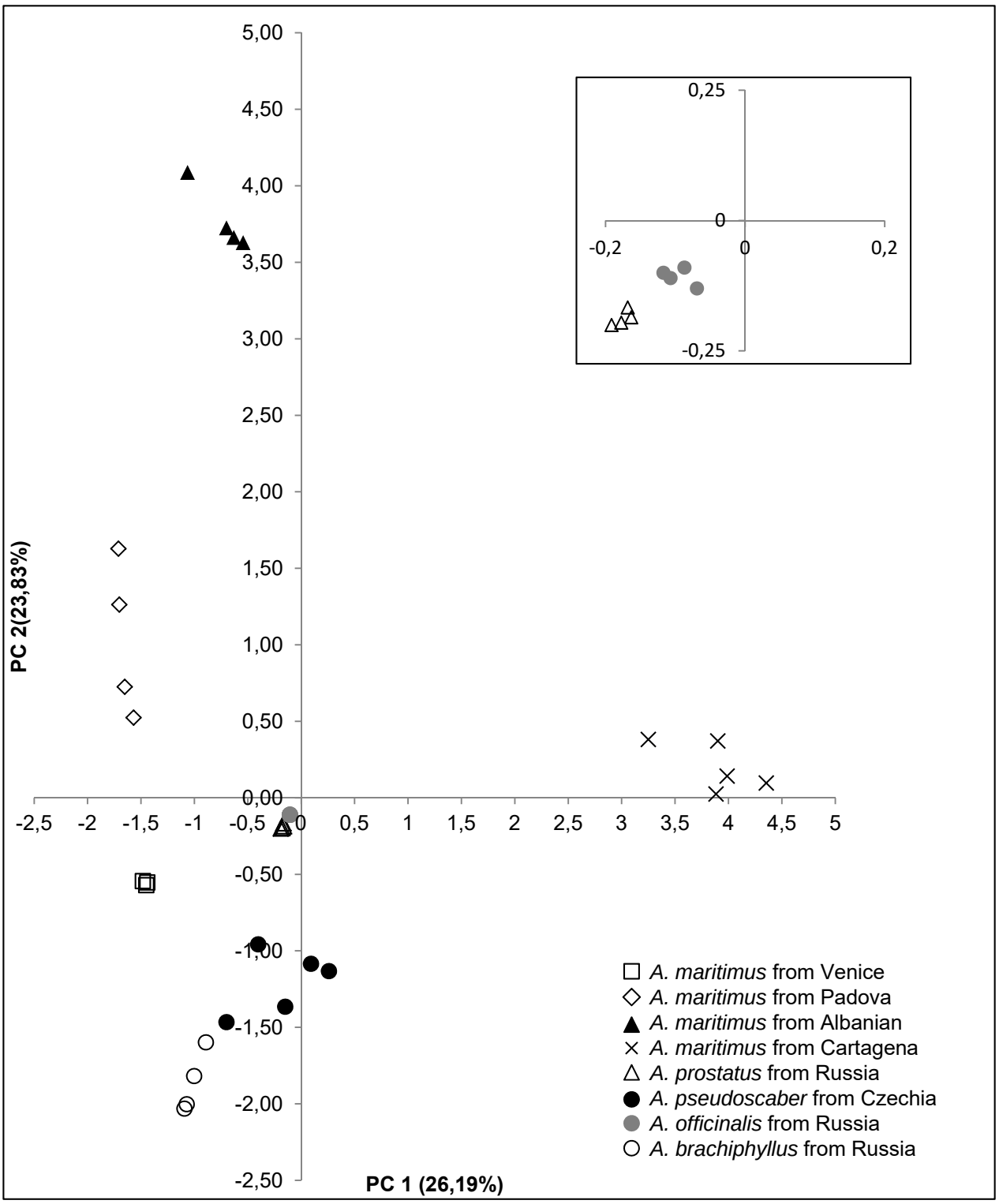


Figure 5 Principal component analyses of saponin profiles of different wild asparagus samples from different seed origin (n=35).



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