Identification and Structural Analysis of Spirostanol Saponin from *Yucca schidigera* by Integrating Silica Gel Column Chromatography and Liquid Chromatography/Mass Spectrometry Analysis

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Abstract: *Yucca schidigera* Roezl (Mojave), a kind of ornamental plant belonging to the *Yucca* genus (Agavaceae), whose extract exhibits important roles in food, beverage, cosmetic and feed additives owing to its rich spirostanol saponins. To provide a comprehensive chemical profiling of the spirostanol saponins in it, this study was performed by using a multi-phase liquid chromatography method combining a reversed phase chromatography T₃ column with a normal phase chromatography silica column for the separation and an ESI-Q-Exactive-Orbitrap MS in positive ion mode as the detector. By comparing the retention time and ion fragments with standards, thirty-one spirostanol saponins were identified. In addition, according to the summary of the chromatographic retention behaviors and the MS/MS cleavage patterns and biosynthetic pathway, another seventy-nine spirostanol saponins were speculatively identified, forty ones of which were potentially new ones. Moreover, ten novel spirostanol saponins (three pairs of (25R/S)-spirostanol saponin isomer mixtures) were targeted for isolation to verify the speculation. Then, the comprehensive chemical profiling of spirostanol saponins from *Y. schidigera* was reported here firstly.

Keywords: *Yucca schidigera* Roezl (Mojave); spirostanol saponins; chemical constituents profiling; multi-phase liquid chromatography; normal phase chromatography silica column separation; reversed phase liquid chromatography/mass spectrometry analysis

1. Introduction

*Yucca schidigera* Roezl (Mojave), belonging to the genus *Yucca*, Agavaceae family, is mainly distributed in the southwestern United States and the northern deserts of Mexico [1]. It is a commonly used commercial raw material for steroid saponins, which mainly contains spirostanol, isospirostanol and furostanol saponins. Modern pharmacological studies have shown that the extract of *Y. schidigera* possessed...
many bioactivities, such as regulating energy metabolism and improving animal breeding [2,3]. Therefore, it has been widely used in foods, feeds, and cosmetic additives [4]. According to the references, pharmacological studies were mainly focused on its extracts, while the identity of its effective substance(s) is not clear yet since the lack of systematic phytochemistry studies. Furthermore, the lack of quality assessment standard has limited its development and utilization as well [5], which indicated that a comprehensive chemical profiling of Y. schidigera was urgently needed.

In order to solve this problem, preliminary studies of the chemical composition of this plant have been conducted in our lab [1,6], and a number of standards for our follow-up research have been prepared. Besides, although liquid chromatography-mass spectrometry (LC-MS) seems to be a good choice for chemical composition characterization, the complexity of plant ingredients always leads to some ignorance about low-level substances, which would result in a chemical composition analysis far from comprehensive [7,8]. Thus, according to the literature, a strategy using multi-phase liquid chromatography (MPLC) combined with MS was considered to improve the chromatographic peak capacity [9].

In this paper, an extract of Y. schidigera was separated by a reversed phase chromatography T₃ column after pretreating with a normal phase chromatography silica column, then analyzed by an ESI-Q-Exactive-Orbitrap MS in positive-ion mode. At the same time, the chromatographic retention behaviors and MS/MS cleavage patterns were summarized. In the light of these summaries, 110 spirostanol saponins were identified in the extract by the MPLC-MS method, and a comprehensive characterization of its spirostanol saponins was set up. Then the verification of the characterizations was accomplished by targeted isolation of ten novel spirostanol saponins (including three pairs of them being (25R/S)-spirostanol saponin isomer mixtures).

2. Results and Discussion

Through comparing the separation effects of different columns (HSS C₁₈, T₃, CSH FP and BEH C₁₈), mobile phase compositions (MeOH-H₂O, ACN-H₂O, and MeOH-ACN-H₂O), pH values (0.1% FA and 0.01% FA), and the column temperatures (30 °C and 35 °C) (Figures S1–S4), the optimal analytical condition for UHPLC was determined as shown in Section 3.2.3. As the composition of YS is complex, in order to solve the severely overlapping chromatographic peaks, the YS extract was pre-treated with a normal-phase silica gel column before analyzing with the T₃ column (Figure 1).
Figure 1. BPC for YS, and standard references 5, 7, 16, 17, 26, 30–33, 35, 37, 39, 44, 46, 47, 52, 56, 57, 60, 61, 66, 72, 75, 83–86, 97, 100, 104, 105 by UHPLC-ESI-Q-Exactive-Orbitrap MS/MS in positive ion mode. Column: Waters ACQUITY UPLC® T3 (2.1 x 100 mm, 1.8 μm). Mobile phase: H2O (A) and FA-MeOH-ACN (0.1:50:50, v/v/v) (B). Gradient program: 0–15 min, 12–24% B; 15–30 min, 24–32% B; 30–42 min, 32–40% B; 42–47 min, 40–60% B; 47–65 min, 60–95% B. Flow rate: 0.3 mL/min. Column temperature: 30 °C. Injection volume: 3 μL. ESI-Q-Exactive-Orbitrap MS mode: positive ion mode.
2.1. **Superiority of MPLC-MS than Direct LC-MS in the Identification of Spirostanol Saponins from YS**

MPLC-MS, combining the advantages of the large peak capacity of MPLC and high sensitivity of mass spectrometry, had a wide range of applications, especially in the systematic chemical composition characterization of TCMs [7,9]. After YS was separated by silica gel column chromatography, the response of some chromatographic peaks was observed to be noticeably improved and some trace peaks were enriched (Figure S5).

2.1.1. Separation and Selectivity of MCI and Silica Gel Column

Through preliminary analysis, the saponins of *Y. schidigera* were mainly seen in the silica gel fractions 6–9 of YS (YSESs6–9) and MCI gel fractions 7–9 of YS (YSEMs7–9). The Sieve statistical results indicated that YSEMs7–9 possessed less peaks than YSESs6–9 (YSEMs7–9: 1024 peaks detected; YSESs6–9: 1301 peaks detected). Besides, for example, the ion peak of \( m/z \ 741.4420 \) could be found in the BPC of YSES6, but not in YSEMs7–9 (Figure S6). This result suggested that MPLC-MS technique using silica gel column chromatography as a preliminary separation method could result in a better orthogonal effect than MCI.

2.1.2. Separation and Selectivity of MCI and Silica Gel Column

The Sieve statistical results indicated that MPLC-MS using silica gel chromatography as preliminary separation method resulted in many more peaks than direct LC-MS (YS: 486 peaks detected; YSESs6–9: 1301 peaks detected). For example, by comparing the extracted ion chromatograms of \( m/z \ 755.4223 \) in YS and YSES6, it could be indicated that (25R)-Yucca spirostanoside E4 (37.76 min, \( m/z \ 755.4223 \)) was only found in the silica gel column separation extract (Figure S7).

2.2. **Structural Elucidation of Spirostanol Saponins from YSESs by MPLC-MS**

2.2.1. Structural Identification of Known Spirostanol Saponins by Comparing with Standard References

Through comparing with standard references (Figure S8), peaks 5, 7, 16, 17, 26, 30–33, 35, 37, 39, 44, 46, 47, 52, 56, 57, 60, 61, 66, 72, 75, 83–86, 97, 100, 104, 105 were unambiguously identified (Table 1).
Table 1. The qualitative analysis of compounds 1–110 by ESI-Q-Exactive-Orbitrap MS

| No. | t_R (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z (Intensity)) | Identification Method |
|-----|-----------|----------|---------|-------------|-----------|--------|-----|-------------------------|----------------------|
| 1   | 15.85     | 5β-Spirost-25(27)-en-3β-ol-12-one 3-O-Glc(1→6)-[Glc(1→2)-Glc(1→3)]-Glc | C_{45}H_{72}O_{19} | [M + H]^+ | 885.4478 | 885.4502 | 2.71 | 885.4502 (6.93), 753.4117 (1.01), 591.3535 (2.01), 429.3017 (100.00), 411.2912 (25.80), 393.2797 (13.61), 379.3004 (3.75), 285.2583 (1.68), 273.2222 (62.25), 255.2116 (100.00) | MS/MS |
| 2   | 16.58     | 5β-Spirost-25(27)-en-3β-ol-12-one 3-O-Glc(1→6)-[Glc(1→2)-Xyl(1→3)]-Glc | C_{50}H_{80}O_{22} | [M + H]^+ | 1033.5211 | 1033.5211 | 0.00 | 1033.5211 (1.55), 871.4718 (3.70), 577.3736 (2.29), 415.3219 (25.80), 397.3115 (13.61), 379.3004 (3.75), 285.2583 (1.68), 273.2222 (62.25), 255.2116 (100.00) | MS/MS |
| 3   | 19.76     | 5β-Spirost-25(27)-en-3β-ol-12-one 3-O-Api(1→3)-[Glc(1→2)]-Gal | C_{44}H_{68}O_{18} | [M + H]^+ | 887.4635 | 887.4622 | −1.46 | 887.4622 (1.10), 869.4547 (0.73), 755.4313 (0.44), 593.3715 (1.55), 575.3571 (0.21), 431.3175 (15.74), 413.3065 (18.44), 395.2967 (8.47), 377.2862 (2.53), 301.2543 (5.30), 289.2170 (16.59), 281.2267 (1.00), 269.1989 (12.9) | MS/MS |
| 4   | 21.16     | 5β-Spirost-25(27)-en-3β,12β-diol 3-O-Xyl(1→3)-[Glc(1→2)]-Gal | C_{44}H_{68}O_{18} | [M + H]^+ | 901.4428 | 901.4410 | −2.00 | 901.4410 (0.45), 740.1865 (0.34), 607.3474 (4.46), 577.3405 (0.80), 445.2951 (100.00), 427.2850 (15.40), 409.2734 (8.60), 391.2632 (3.36), 373.2473 (0.67), 333.2343 (3.12), 315.2321 (6.24), 302.0273 (0.38), 297.2220 (5.30), 285.1847 (1.26), 279.2111 (1.03), 269.1965 (0.75) | MS/MS, standard reported by literature [1] |
| 5   | 21.19     | Yucca spirostanoside D_1 | C_{44}H_{68}O_{19} | [M + H]^+ | 917.4741 | 917.4745 | 0.44 | 917.4745 (1.77), 899.4671 (1.87), 755.4432 (1.38), 593.3698 (7.14), 431.3167 (19.21), 413.3065 (100.00), 395.2967 (10.11), 377.2853 (1.18), 301.2537 (3.22), 289.2166 (15.64), 283.2431 (9.17), 271.2016 (15.46), 253.1956 (6.79) | MS/MS, standard reported by literature [1] |
| 6   | 21.73     | 5β-Spirost-25(27)-en-3β-ol-12-one 3-O-Api(1→3)-[Glc(1→2)]-Glc | C_{44}H_{68}O_{18} | [M + H]^+ | 885.4478 | 885.4480 | 0.23 | 885.4480 (3.83), 753.4117 (1.01), 591.3535 (2.01), 429.3017 (100.00), 411.2912 (25.80), 393.2797 (13.61), 377.2862 (2.53), 301.2543 (5.30), 289.2170 (16.59), 281.2267 (1.00), 269.1989 (12.9) | MS/MS |
| 7   | 22.24     | Yucca spirostanoside B_3 | C_{45}H_{72}O_{19} | [M + H]^+ | 917.4741 | 917.4745 | 0.44 | 917.4745 (1.77), 899.4671 (1.87), 755.4432 (1.38), 593.3698 (7.14), 431.3167 (19.21), 413.3065 (100.00), 395.2967 (10.11), 377.2853 (1.18), 301.2537 (3.22), 289.2166 (15.64), 283.2431 (9.17), 271.2016 (15.46), 253.1956 (6.79) | MS/MS, standard reported by literature [1] |
Table 1. Cont.

| No. | \( t_R \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|-----------------|----------|---------|-------------|-----------|--------|-----|---------------------|----------------------|
| 8   | 22.63           | 25(S):5\(\beta\)-Spirostan-3\(\beta\),12\(\beta\)-diol 3-O-Glc\(1\rightarrow 3\)-Glc\(1\rightarrow 2\)]-Glc \( ^8 \) | \( C_{46}H_{74}O_{19} \) | \([M + H]^+ \) | 919.4897 | 919.4919 | 2.39 | 900.4546 (0.88), 739.4276 (3.67), 595.3859 (5.33), 577.3771 (4.10), 433.3300 (100.00), 415.3225 (25.37), 397.3117 (7.05), 379.3006 (0.88), 301.2531 (2.67), 289.2171 (7.64), 283.2430 (11.85), 271.2071 (6.41), 253.1956 (4.02) | MS/MS |
| 9   | 22.99           | YS XII   | \( C_{45}H_{74}O_{19} \) | \([M + H]^+ \) | 919.4897 | 919.4919 | 2.39 | 900.4546 (0.88), 739.4276 (3.67), 595.3859 (5.33), 577.3771 (4.10), 433.3300 (100.00), 415.3225 (25.37), 397.3117 (7.05), 379.3006 (0.88), 301.2531 (2.67), 289.2171 (7.64), 283.2430 (11.85), 271.2071 (6.41), 253.1956 (4.02) | MS/MS, literature [10] |
| 10  | 23.04           | 25(\(R\):5\(\beta\)-Spirostan-2\(\beta\),3\(\beta\)-ol-12-one-3-O- Xyl\(1\rightarrow 3\)-Glc\(1\rightarrow 2\)]-Gal | \( C_{44}H_{70}O_{19} \) | \([M + H]^+ \) | 903.4584 | 903.4550 | -3.76 | 609.3638 (2.30), 447.3109 (100.00), 429.3012 (23.67), 411.2903 (16.39), 393.2797 (2.64), 333.2435 (4.23), 315.2324 (14.86), 299.2218 (14.03), 285.1842 (1.55), 279.2117 (4.11) | MS/MS |
| 11  | 23.16           | 5\(\beta\)-Spirost-25(27)-en-3\(\beta\)-ol-12-one-3-O- Xyl\(1\rightarrow 3\)-Glc\(1\rightarrow 2\)]-Gal | \( C_{44}H_{68}O_{19} \) | \([M + H]^+ \) | 885.4512 | 885.4512 | 3.84 | 885.4512 (21.56), 753.4061 (13.27), 591.3555 (11.51), 429.3015 (100.00), 411.2905 (18.53), 393.2809 (8.23), 375.2710 (13.33), 299.2365 (17.25), 281.2261 (11.89) | MS/MS, literature [11] |
| 12  | 23.32           | 5\(\beta\)-Spirost-25(27)-en-3\(\beta\),12\(\beta\)-diol 3-O-Xyl\(1\rightarrow 3\)-Glc\(1\rightarrow 2\)]-Gal \( ^8 \) | \( C_{44}H_{70}O_{19} \) | \([M + H]^+ \) | 887.4638 | 887.4638 | 0.34 | 887.4638 (2.66), 869.4570 (1.20), 755.4313 (0.59), 593.3729 (1.19), 431.3169 (16.33), 413.3061 (16.54), 395.2949 (7.13), 377.2860 (0.72), 301.2534 (8.14), 289.2169 (17.14), 283.2429 (100.00), 271.2065 (16.39), 253.1966 (7.32) | MS/MS |
| 13  | 23.95           | 25(\(R\)/\(S\):5\(\beta\)-Spirostan-3\(\beta\),12\(\beta\)-diol 3-O-Xyl\(1\rightarrow 3\)-Glc\(1\rightarrow 2\)]-Gal \( ^8 \) | \( C_{44}H_{72}O_{19} \) | \([M + H]^+ \) | 889.4826 | 889.4826 | 2.93 | 889.4826 (0.25), 757.4467 (0.67), 595.3866 (0.82), 433.3326 (20.48), 415.3224 (100.00), 397.3108 (8.28), 379.2990 (1.29), 301.2537 (9.22), 289.2169 (14.84), 283.2430 (21.20), 271.2068 (15.87), 253.1960 (8.47) | MS/MS |
| 14  | 24.52           | 25(\(S\):5\(\beta\)-Spirostan-3\(\beta\),12\(\beta\)-diol 3-O-Xyl\(1\rightarrow 3\)-Glc\(1\rightarrow 2\)]-Gal | \( C_{44}H_{72}O_{19} \) | \([M + H]^+ \) | 889.4791 | 889.4772 | -2.14 | 889.4772 (5.85), 757.4364 (1.66), 595.3867 (7.05), 433.3323 (77.23), 415.3209 (93.33), 379.3113 (35.91), 379.2998 (7.35), 301.2537 (37.53), 289.2171 (64.86), 283.2435 (100.00), 271.2072 (71.97), 253.1954 (36.78) | MS/MS |
| No. | $t_R$ (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|-------------|----------|---------|-------------|-----------|--------|------|-----------------------|-----------------------|
| 15  | 24.54       | 25(R)-5β-Spirostan-3β,12β-diol 3-0-Xyl(1→3)-[Glc(1→2)]-Glc | C$_{44}$H$_{72}$O$_{18}$ | [M + H]$^+$ | 889.4791 | 889.4767 | -2.70 | 889.4767 (7.24), 757.4364 (1.66), 595.3850 (5.81), 433.3330 (87.31), 415.3299 (100.00), 397.3113 (39.92), 379.2998 (18.12), 301.2537 (58.12), 269.2171 (73.16), 283.2435 (98.22), 271.2072 (78.89), 253.1954 (35.94) | MS/MS |
| 16  | 24.76       | Yucca spirostanoside C$_3$ | C$_{45}$H$_{70}$O$_{19}$ | [M + H]$^+$ | 915.4584 | 915.4576 | -0.87 | 915.4576 (5.20), 753.4245 (14.19), 591.3542 (3.79), 429.3012 (100.00), 411.2907 (23.96), 393.2802 (10.50), 299.2384 (14.06), 281.2284 (8.73), 269.1950 (1.20) | MS/MS, standard reported by literature [1] |
| 17  | 26.12       | 5β-Spirost-25(27)-en-3β-ol-12-one-3-0-Glc(1→2)-O-[Glc(1→3)]-Glc | C$_{45}$H$_{70}$O$_{19}$ | [M + H]$^+$ | 915.4584 | 915.4620 | 3.93 | 915.4620 (3.29), 753.4156 (5.16), 591.3542 (4.97), 429.3015 (100.00), 411.2907 (23.01), 393.2802 (10.50), 299.2384 (14.06), 281.2284 (8.73), 269.1950 (1.28) | MS/MS, standard reported by literature [1] |
| 18  | 26.26       | (25R/S)-5β-Spirostan-3β-ol-12-one-3-0-Glc(1→2)-[Glc(1→3)]-Gal | C$_{45}$H$_{70}$O$_{19}$ | [M + H]$^+$ | 917.4741 | 917.4763 | 2.40 | 889.4560 (1.08), 755.4058 (1.02), 593.3698 (7.14), 431.3166 (100.00), 413.3060 (21.21), 395.2961 (10.92), 377.2844 (3.01), 299.2372 (8.79), 281.2269 (6.81), 269.1903 (3.22) | MS/MS |
| 19  | 27.06       | (25R/S)-5β-Spirostan-3β-ol-12-one-3-0-Xyl(1→3)-[Glc(1→2)]-Gal | C$_{44}$H$_{68}$O$_{18}$ | [M + H]$^+$ | 889.4766 | 889.4766 | -2.81 | 889.4766 (4.20), 757.4364 (1.66), 595.3846 (4.34), 433.3315 (44.14), 415.3234 (62.06), 397.3110 (100.00), 379.3011 (11.82), 301.2541 (3.38), 287.2002 (1.54), 281.2266 (2.64), 269.1928 (0.24) | MS/MS |
| 20  | 27.20       | Schidigera-saponin B$_1$ | C$_{44}$H$_{68}$O$_{18}$ | [M + H]$^+$ | 885.4478 | 885.4485 | 0.79 | 885.4485 (13.49), 751.3990 (4.73), 591.3539 (8.27), 429.3008 (100.00), 411.2915 (28.92), 393.2981 (13.93), 317.2474 (1.50), 299.2379 (9.89), 287.2002 (1.54), 281.2266 (2.64), 269.1928 (0.24) | MS/MS, literature [5] |
| 21  | 27.27       | (25S)-3β-{[(O-Glc(1→2)]-Gallyoxy}-5β-spirostan-12-one | C$_{39}$H$_{62}$O$_{14}$ | [M + H]$^+$ | 755.4212 | 755.4200 | -1.59 | 755.4200 (1.45), 737.4117 (2.38), 719.3984 (2.09), 593.3697 (3.68), 575.3598 (0.97), 431.3176 (20.32), 413.3061 (16.68), 395.2953 (100.00), 377.2847 (8.81), 317.2493 (2.66), 299.2377 (12.94), 281.2273 (8.58) | MS/MS |
| 22  | 27.28       | (25R)-3β-{[(O-Glc(1→2)]-Gallyoxy}-5β-spirostan-12-one | C$_{39}$H$_{62}$O$_{14}$ | [M + H]$^+$ | 755.4212 | 755.4213 | 0.13 | 755.4213 (2.68), 737.4111 (3.76), 719.3994 (1.47), 593.3711 (10.20), 575.3606 (2.35), 431.3175 (44.57), 413.3069 (100.00), 395.2958 (18.65), 377.2850 (6.00), 317.2488 (6.49), 299.2379 (22.27), 281.2273 (15.26) | MS/MS, literature [12] |
Table 1. Cont.

| No. | \( t_R \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|-----------------|----------|---------|-------------|-----------|-------|-----|---------------------|---------------------|
| 23  | 27.47           | (2S,5S)-Spirostan-3β-ol-12-one 3-O-Xyl(1→3)[Glc(1→2)]-Gal * | C\(_{44}H_{70}O_{18}\) | [M + H]** | 887.4635 | 887.4618 | -1.92 | 887.4618 (5.66), 755.4180 (2.07), 593.3669 (6.91), 431.3166 (100.00), 413.3072 (26.31), 395.2953 (17.04), 377.2848 (3.09), 299.2283 (7.90), 281.2273 (6.10) | MS/MS |
| 24  | 27.88           | (2R,5R)-Spirostan-3β-ol-12-one 3-O-Xyl(1→3)[Glc(1→2)]-Gal | C\(_{44}H_{70}O_{18}\) | [M + H]** | 887.4635 | 887.4619 | -1.80 | 887.4619 (5.56), 755.4180 (3.35), 593.3671 (6.77), 431.3168 (77.21), 413.3070 (100.00), 395.2954 (18.08), 377.2849 (3.11), 299.2383 (7.95), 281.2275 (6.70) | MS/MS, literature [11] |
| 25  | 28.16           | 5β-Spirost-25(27)-en-3β-ol-12-one 3-O-Glc(1→6)[Glc(1→3)]-Glc | C\(_{45}H_{72}O_{19}\) | [M + H]** | 915.4584 | 915.4617 | 3.60 | 899.4603 (0.63), 755.4173 (0.55), 593.3710 (9.97), 431.3166 (100.00), 413.3062 (23.14), 395.2959 (14.93), 377.2847 (2.67), 299.2280 (2.68), 269.1909 (1.93) | MS/MS |
| 26  | 28.75           | YS-VII * | C\(_{45}H_{72}O_{19}\) | [M + H]** | 917.4741 | 917.4773 | 3.49 | MS/MS, standard isolated by our lab |
| 27  | 28.81           | (2R,5S)-Spirostan-3β-ol-12-one 3-O-β-Api(1→3)-[Glc(1→2)]-Glc | C\(_{44}H_{70}O_{18}\) | [M + H]** | 887.4635 | 887.4618 | -1.92 | 887.4618 (2.26), 755.4180 (2.07), 593.3669 (6.91), 431.3166 (100.00), 413.3072 (26.31), 395.2953 (17.04), 377.2848 (3.09), 299.2283 (7.90), 281.2273 (6.10) | MS/MS |
| 28  | 28.83           | 25(\(R\)/\(S\))-Spirostan-3β,12β-diol 3-O-Xyl(1→3)-Gal | C\(_{38}H_{62}O_{13}\) | [M + H]** | 727.4263 | 727.4234 | -3.99 | 727.4234 (19.44), 433.3307 (5.31), 415.3207 (18.74), 397.3099 (3.86), 301.2521 (20.65), 289.2165 (14.43), 283.2428 (100.00), 271.2067 (22.74), 253.1959 (6.20) | MS/MS |
| 29  | 28.85           | 25(\(R\)/\(S\))-Spirostan-1β,3β-diol 3-O-Xyl(1→3)-[Glc(1→2)]-Glc | C\(_{44}H_{72}O_{18}\) | [M + H]** | 889.4791 | 889.4826 | 3.93 | 889.4826 (4.20), 755.4180 (1.66), 593.3669 (3.42), 431.3222 (37.49), 413.3223 (37.80), 397.3106 (100.00), 379.3000 (12.21), 283.2428 (19.47), 271.2067 (27.54), 253.1958 (36.41) | MS/MS |
| 30  | 29.24           | (25S)-Yucca spirostanoside E1 * | C\(_{39}H_{62}O_{14}\) | [M + H]** | 755.4212 | 755.4240 | 3.71 | 755.4240 (1.74), 737.4130 (6.61), 719.4015 (2.78), 593.3713 (9.00), 575.3615 (2.97), 431.3175 (100.00), 413.3065 (20.10), 395.2957 (14.54), 377.2835 (5.25), 317.2497 (6.17), 299.2381 (20.64), 281.2272 (14.25) | MS/MS, standard reproted by literature [6] |
Table 1. Cont.

| No. | $t_R$ (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff  | MS/MS (m/z) (Intensity) | Identification Method |
|-----|-------------|----------|---------|-------------|-----------|--------|------|------------------------|----------------------|
| 31  | 29.42       | (25R)-Yucca spirostanoside E$_3$ * | C$_{39}$H$_{62}$O$_{14}$ | [M + H]$^+$ | 755.4212 | 755.4202 | −1.32 | 755.4202 (2.79), 737.4119 (2.61), 719.4024 (2.75), 593.3701 (9.37), 575.3682 (2.56), 431.3171 (100.00), 413.3065 (24.54), 395.2972 (16.95), 377.2855 (5.97), 317.2495 (7.55), 299.2380 (27.91), 281.2278 (19.49) | MS/MS, standard reported by literature [6] |
| 32  | 29.51       | 25(S)-Schidigera-saponin E$_1$ * | C$_{44}$H$_{70}$O$_{18}$ | [M + H]$^+$ | 887.4635 | 887.4651 | 1.80  | 887.4651 (2.71), 869.4508 (1.62), 755.4108 (1.28), 593.3730 (8.57), 431.3166 (100.00), 413.3054 (30.71), 395.2961 (15.95), 377.2864 (1.86), 299.2373 (14.75), 281.2265 (10.52), 269.1911 (1.89) | MS/MS, standard isolated by our lab |
| 33  | 30.30       | Yucca spirostanoside B$_2$ * | C$_{38}$H$_{60}$O$_{13}$ | [M + H]$^+$ | 725.4107 | 725.4112 | 0.69  | 725.4112 (21.14), 707.4166 (0.28), 593.3740 (2.47), 431.3172 (7.77), 413.3062 (22.17), 395.2972 (16.95), 377.2859 (8.19), 301.2539 (26.06), 289.2173 (36.03), 283.2430 (100.00), 271.2065 (38.65), 253.1959 (24.28) | MS/MS, standard reported by literature [1] |
| 34  | 31.75       | 25(S)-5β-Spirostan-3β,12β-diol 3-O-Xyl(1→3)-Glc | C$_{38}$H$_{62}$O$_{13}$ | [M + H]$^+$ | 727.4263 | 727.4299 | 4.95  | 727.4299 (34.55), 595.3719 (2.78), 577.3777 (2.50), 433.3330 (12.89), 415.3212 (20.56), 397.3104 (11.37), 379.3010 (4.10), 301.2542 (26.06), 289.2173 (36.03), 283.2430 (100.00), 271.2065 (38.65), 253.1959 (24.28) | MS/MS |
| 35  | 31.79       | Yucca spirostanoside B$_1$ * | C$_{39}$H$_{62}$O$_{15}$ | [M + H]$^+$ | 593.3684 | 593.3701 | 2.86  | 593.3701 (58.75), 575.3610 (15.96), 539.3178 (3.66), 431.3172 (7.77), 395.2964 (19.97), 377.2858 (9.63), 301.2532 (16.35), 289.2174 (42.46), 283.2431 (100.00), 271.2067 (61.06), 253.1960 (30.48) | MS/MS, standard reported by literature [1] |
| 36  | 32.01       | 25(R)-5β-Spirostan-3β,12β-diol 3-O-Xyl(1→3)-Glc | C$_{38}$H$_{62}$O$_{13}$ | [M + H]$^+$ | 727.4263 | 727.4261 | −0.27 | 727.4261 (39.39), 433.3314 (17.80), 415.3228 (26.74), 397.3110 (24.64), 379.3010 (4.10), 301.2542 (26.06), 289.2173 (36.03), 283.2430 (100.00), 271.2067 (61.06), 253.1959 (8.24) | MS/MS |
| 37  | 32.51       | Schidigera-saponin C$_1$ * | C$_{44}$H$_{70}$O$_{18}$ | [M + H]$^+$ | 887.4635 | 887.4665 | 3.38  | 887.4665 (2.75), 755.4108 (1.41), 593.3685 (3.95), 431.3171 (100.00), 413.3065 (45.15), 395.2947 (9.03), 377.2824 (1.43), 397.2971 (70.00), 283.2418 (1.37), 271.2067 (100.00), 253.1960 (37.96) | MS/MS, standard reported by literature [1] |
| 38  | 32.51       | (25R)-5β-Spirostan-3β-ol-12-one 3-O-Xyl(1→3)-[Glc(1→6)]-Glc | C$_{44}$H$_{70}$O$_{18}$ | [M + H]$^+$ | 887.4635 | 887.4665 | 3.38  | 593.3695 (8.59), 539.3172 (100.00), 431.3065 (45.15), 395.2947 (18.25), 377.2838 (4.77), 299.2377 (8.97), 281.2274 (8.71), 269.1907 (3.33) | MS/MS |
| No. | $t_R$ (min) | Compound | Formula | Adduct Ions | Theoretic Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|-------------|-----------|---------|-------------|------------------|-----|------------------------|---------------------|
| 39  | 34.06       | Schidigera-saponin C₂ * | C₃₀H₴₅O₁₄ | [M + H]$^+$ | 755.4212 | 755.4232 | 2.65 | 755.4232 (3.07), 593.3716 (1.20), 431.3171 (21.93), 413.3065 (33.67), 395.2959 (7.00), 377.2863 (1.18), 289.2172 (60.83), 269.1923 (0.67), 271.2067 (100.00), 253.1959 (24.93) | MS/MS, standard reported by literature [1] |
| 40  | 33.51       | 25(5)-5β-Spirostan-3β,12β-diol 3-O-Glc | C₃₁H₴₄O₉ | [M + H]$^+$ | 595.3841 | 595.3864 | 3.86 | 595.3864 (68.74), 433.2562 (1.99), 397.3130 (3.88), 379.3032 (4.41), 301.2534 (23.47), 289.2170 (16.30), 283.2432 (100.00), 271.2061 (23.39), 253.1955 (14.22) | MS/MS |
| 41  | 33.69       | 25(5)-5β-Spirostan-3β,12β-diol 3-O-Glc | C₃₁H₴₄O₉ | [M + H]$^+$ | 595.3841 | 595.3864 | 3.86 | 595.3864 (72.38), 433.2563 (1.99), 397.3130 (3.88), 379.3032 (4.41), 301.2546 (19.14), 289.2170 (14.86), 283.2429 (100.00), 271.2065 (35.10), 253.1959 (16.57) | MS/MS |
| 42  | 32.47       | 5β-Spirost-25(27)-en-2β,3β-diol 3-O-Xyl(1→3)-Gal $^a$ | C₃₈H₅₀O₁₃ | [M + H]$^+$ | 725.4107 | 725.4124 | 2.34 | 725.4124 (3.41), 707.4037 (1.07), 593.3687 (4.44), 431.3184 (39.43), 413.3065 (61.00), 395.2963 (23.55), 289.2172 (100.00), 283.2428 (6.71), 271.2066 (73.42), 253.1959 (33.59) | MS/MS |
| 43  | 34.57       | 5β-Spirost-25(27)-en-3β-ol-12-one 3-O-Glc(1→3)-Glc $^a$ | C₃₉H₆₀O₁₄ | [M + H]$^+$ | 753.4056 | 753.4091 | 4.56 | 753.4091 (17.55), 591.3540 (15.97), 429.3008 (100.00), 411.2898 (30.12), 393.2813 (21.08), 299.2374 (32.01), 281.2271 (18.84) | MS/MS |
| 44  | 35.94       | 25(S)-Schidigera-saponin F₁ $^b$ | C₄₄H₇₂O₁₈ | [M + H]$^+$ | 889.4791 | 889.4822 | 3.49 | 889.4822 (4.20), 757.4346 (1.66), 595.3850 (5.93), 433.3232 (100.00), 415.3212 (46.55), 397.3115 (9.38), 379.3004 (21.5), 301.2536 (2.40), 289.2171 (65.36), 283.2439 (3.56), 271.2063 (71.42), 253.1959 (34.71) | MS/MS, standard isolated by our lab |
| 45  | 36.36       | 25(R)-Schidigera-saponin F₁ | C₄₄H₇₂O₁₈ | [M + H]$^+$ | 889.4791 | 889.4821 | 3.37 | 889.4821 (4.20), 757.4346 (1.66), 595.3850 (5.93), 433.3232 (100.00), 415.3225 (67.62), 397.3102 (8.19), 379.3004 (21.8), 301.2536 (2.40), 289.2170 (62.53), 283.2441 (3.60), 271.2064 (63.65), 253.1961 (31.42) | MS/MS, literature [5] |
| 46  | 36.77       | Yucca spirostanoside C₂ $^b$ | C₃₈H₅₈O₁₃ | [M + H]$^+$ | 723.3950 | 723.3977 | 3.73 | 723.3977 (4.31), 705.3864 (12.41), 687.3758 (6.45), 591.3540 (12.47), 573.3441 (10.91), 555.3307 (1.61), 429.3013 (43.67), 411.2910 (54.19), 393.2802 (44.17), 375.2698 (19.47), 317.2483 (18.16), 299.2378 (19.00), 281.2270 (41.92), 269.1910 (9.61), 251.1804 (3.87) | MS/MS, standard reported by literature [1] |
Table 1. Cont.

| No. | $r_\text{k}$ (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|------------------|----------|---------|-------------|-----------|--------|-----|-------------------------|----------------------|
| 47  | 37.37            | 25(S)-Schidigera-saponin F * | C$_{39}$H$_{64}$O$_{14}$ | [M + H]$^+$ | 757.4369 | 757.4332 | -2.79 | 757.4332 (1.82), 595.3813 (2.03), 433.3332 (26.30), 415.3218 (39.12), 397.3110 (9.34), 301.2543 (2.12), 289.2172 (100.00), 263.2439 (2.92), 271.2066 (62.23), 253.1962 (27.30) | MS/MS, standard isolated by our lab |
| 48  | 37.47            | (25S)-Spirostan-3β-ol-12-one 3-O-Xyl(1→3)-Gal $^f$ | C$_{38}$H$_{60}$O$_{13}$ | [M + H]$^+$ | 725.4107 | 725.4122 | 2.07 | 725.4122 (14.77), 707.4008 (11.22), 593.3725 (19.80), 575.3577 (4.45), 431.3171 (100.00), 413.3061 (51.82), 395.2970 (26.36), 377.2819 (2.98), 299.2374 (34.86), 281.2279 (18.57), 269.1892 (2.30) | MS/MS |
| 49  | 37.66            | (25R)-Spirostan-3β-ol-12-one 3-O-Xyl(1→3)-Gal $^f$ | C$_{38}$H$_{60}$O$_{13}$ | [M + H]$^+$ | 725.4107 | 725.4090 | -0.40 | 725.4090 (8.00), 707.4029 (20.20), 593.3709 (19.76), 575.3591 (8.89), 431.3179 (100.00), 413.3059 (42.51), 395.2968 (38.05), 377.2858 (14.82), 299.2380 (62.72), 281.2271 (39.04), 269.1911 (7.12) | MS/MS |
| 50  | 37.77            | 25(R/S)-Schidigera-saponin F | C$_{39}$H$_{64}$O$_{14}$ | [M + H]$^+$ | 757.4369 | 757.4366 | -0.40 | 757.4366 (1.23), 595.3882 (21.35), 433.3328 (27.43), 415.3219 (39.86), 397.3109 (9.03), 301.2545 (1.68), 289.2172 (100.00), 283.2439 (2.75), 271.2066 (58.68), 253.1962 (25.66) | MS/MS, literature [5] |
| 51  | 37.78            | (25S)-Spirostan-3β-ol-12-one 3-O-Glc(1→3)-Glc $^f$ | C$_{39}$H$_{52}$O$_{14}$ | [M + H]$^+$ | 755.4212 | 755.4230 | 2.38 | 755.4230 (9.78), 737.4004 (8.92), 593.3699 (40.52), 431.3174 (100.00), 413.3055 (52.49), 395.2968 (30.39), 377.2894 (6.82), 299.2377 (37.42), 281.2271 (100.00), 281.2271 (47.22), 251.1799 (5.71) | MS/MS |
| 52  | 38.27            | Yucca spirotanoside C$_1$ * | C$_{33}$H$_{50}$O$_{9}$ | [M + H]$^+$ | 591.3528 | 591.3548 | 3.38 | 591.3548 (35.36), 573.3416 (55.79), 555.3334 (23.91), 429.3005 (6.86), 411.2913 (20.78), 393.2804 (36.45), 375.2680 (23.42), 317.2502 (5.55), 299.2381 (100.00), 281.2274 (47.22), 251.1799 (5.71) | MS/MS, standard reported by literature [1] |
| 53  | 38.38            | 25(S)-Spirostan-2β,3β-diol 3-O-Glc(1→3)-Gal $^f$ | C$_{39}$H$_{64}$O$_{14}$ | [M + H]$^+$ | 757.4369 | 757.4352 | -2.24 | 757.4352 (1.76), 595.3810 (4.74), 433.3313 (9.66), 415.3202 (62.66), 397.3110 (10.96), 301.2529 (4.71), 289.2185 (64.58), 283.2425 (4.70), 271.2055 (100.00), 253.1949 (33.87) | MS/MS |
| 54  | 38.50            | 5β-Spirost-25(27)-en-1β,3β-diol 3-O-Xyl(1→3)-Glc $^f$ | C$_{38}$H$_{60}$O$_{13}$ | [M + H]$^+$ | 725.4107 | 725.4122 | 2.07 | 725.4122 (2.94), 707.4010 (5.95), 593.3736 (5.34), 431.3182 (25.61), 413.3066 (29.77), 395.2963 (44.26), 377.2851 (25.24), 289.2184 (1.32), 283.2433 (23.41), 271.2046 (27.92), 253.1958 (100.00) | MS/MS |
| No. | \( t_R \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|-----------------|----------|---------|-------------|-----------|--------|------|------------------------|----------------------|
| 55  | 38.74           | 25(R)-5β-Spirostan-2β,3β-diol 3-O-Glc(1→3)-Gal * | \( C_{39}H_{64}O_{14}\) | \([M + H]^+\)* | 757.4369 | 757.4352 | -2.24 | 757.4352 (1.76), 595.3810 (4.74), 433.3313 (39.86), 415.3202 (62.66), 397.3110 (10.96), 301.2529 (4.71), 289.2158 (64.58), 283.3425 (4.70), 271.2055 (100.00), 253.1949 (33.87) | MS/MS |
| 56  | 39.49           | (25S)-Yucca spirostanoside E2 * | \( C_{38}H_{60}O_{13}\) | \([M + H]^+\)* | 725.4107 | 725.4116 | 1.24 | 725.4116 (9.29), 707.4016 (23.00), 689.3934 (12.09), 593.3711 (26.56), 575.3602 (11.10), 557.3492 (3.64), 431.3180 (65.59), 413.3069 (48.22), 395.2965 (38.06), 377.2856 (14.46), 317.2494 (20.08), 299.2380 (100.00), 281.2272 (41.64), 269.1906 (4.40) | MS/MS, standard reported by literature [6] |
| 57  | 40.03           | (25R)-Yucca spirostanoside E2 * | \( C_{38}H_{60}O_{13}\) | \([M + H]^+\)* | 725.4107 | 725.4116 | 1.24 | 725.4116 (9.02), 707.4017 (22.76), 689.3934 (12.29), 593.3711 (26.66), 575.3603 (10.76), 557.3492 (3.70), 431.3180 (64.79), 413.3068 (48.23), 395.2965 (37.82), 377.2856 (14.26), 317.2494 (19.80), 299.2380 (100.00), 281.2272 (41.49), 269.1906 (4.40) | MS/MS, standard reported by literature [6] |
| 58  | 40.64           | 25(S)-5β-Spirostan-1β,3β-diol 3-O-Xyl(1→3)-Glc * | \( C_{38}H_{62}O_{13}\) | \([M + H]^+\)* | 727.4263 | 727.4291 | 3.85 | 727.4291 (2.08), 709.4142 (5.57), 691.4039 (28.21), 593.3711 (1.55), 575.3603 (3.78), 433.3225 (10.74), 415.3194 (20.52), 397.3095 (100.00), 379.2986 (39.97), 301.2521 (9.45), 289.2150 (6.16), 269.1906 (32.99), 253.1946 (50.87) | MS/MS |
| 59  | 40.83           | 25(R)-5β-Spirostan-1β,3β-diol 3-O-Xyl(1→3)-Glc * | \( C_{38}H_{62}O_{13}\) | \([M + H]^+\)* | 727.4263 | 727.4291 | 3.85 | 727.4291 (2.08), 709.4142 (5.57), 691.4039 (28.21), 593.3711 (1.55), 577.3726 (3.78), 433.3225 (10.74), 415.3194 (20.52), 397.3095 (100.00), 379.2986 (39.97), 301.2521 (9.45), 289.2150 (6.16), 269.1906 (32.99), 253.1946 (50.87) | MS/MS |
| 60  | 40.86           | (25S)-Yucca spirostanoside E1 * | \( C_{33}H_{52}O_{9}\) | \([M + H]^+\)* | 593.3684 | 593.3704 | 3.37 | 593.3704 (100.00), 575.3602 (37.61), 557.3490 (19.57), 433.3152 (2.97), 413.3061 (10.24), 395.2963 (24.86), 377.2859 (29.27), 317.2491 (11.16), 299.2374 (56.10), 281.2276 (36.70), 269.1902 (7.85) | MS/MS, standard reported by literature [6] |
| 61  | 40.90           | (25R)-Yucca spirostanoside E1 * | \( C_{33}H_{52}O_{9}\) | \([M + H]^+\)* | 593.3684 | 593.3701 | 2.86 | 593.3701 (100.00), 575.3604 (36.38), 557.3493 (17.61), 433.3148 (3.33), 413.3063 (10.21), 395.2965 (23.26), 377.2864 (11.69), 317.2491 (12.05), 299.2374 (53.58), 281.2278 (35.90), 269.1901 (8.53) | MS/MS, standard reported by literature [6] |
Table 1. Cont.

| No. | \( t_R \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z1 (Intensity)) | Identification Method |
|-----|-----------------|----------|---------|-------------|-----------|--------|-----|-------------------------|----------------------|
| 62  | 42.57           | 5β-Spirost-25(27)-en-3-O-Glc(1→3)-[Glc(1→2)]-Gal \# | C43H72O16 | [M + H]\(^+\) | 901.4791 | 901.4750 | −4.55 | 901.4750 (0.65), 739.4295 (0.80), 577.3744 (3.54), 415.3223 (25.12), 397.3108 (5.68), 379.2019 (0.59), 273.2218 (41.36), 255.2113 (100.00) | MS/MS |
| 63  | 42.78           | Rhodea sapogenin 3-O-Glc | C33H54O9 | [M + H]\(^+\) | 595.3841 | 595.3860 | 3.19 | 595.3860 (10.71), 577.3752 (8.21), 559.3649 (39.26), 433.2632 (1.68), 415.3235 (3.08), 397.3110 (21.08), 379.3017 (23.52), 301.2536 (2.98), 289.2166 (0.32), 283.2427 (27.79), 271.2063 (19.19), 255.2113 (100.00) | MS/MS, literature [13] |
| 64  | 42.81           | Isorhodea sapogenin 3-O-Glc | C33H54O9 | [M + H]\(^+\) | 595.3841 | 595.3867 | 4.37 | 595.3867 (10.56), 577.3759 (8.42), 559.3651 (39.58), 433.2600 (2.43), 415.3236 (2.96), 397.3111 (22.01), 379.3015 (26.31), 301.2530 (3.19), 289.2188 (0.07), 283.2429 (32.12), 271.2063 (21.55), 253.1958 (100.00) | MS/MS, literature [13] |
| 65  | 43.60           | Schidigera-saponin A\(_2\) | C44H70O17 | [M + H]\(^+\) | 871.4686 | 871.4683 | −0.34 | 871.4683 (5.58), 739.4295 (0.44), 577.3759 (6.59), 397.3117 (10.42), 379.3015 (1.04), 273.2224 (58.89), 255.2119 (100.00) | MS/MS, literature [5] |
| 66  | 44.37           | Schidigera-saponin A\(_2\)* | C45H72O18 | [M + H]\(^+\) | 901.4791 | 901.4806 | 1.66 | 901.4806 (0.39), 739.4223 (0.60), 577.3760 (4.43), 415.3221 (19.02), 397.3107 (5.97), 273.2225 (43.29), 255.2117 (100.00) | MS/MS, standard reproted by literature [1] |
| 67  | 45.70           | 5β-Spirost-25(27)-en-3-O-Api(1→3)-[Glc(1→2)]-Glc \# | C44H70O17 | [M + H]\(^+\) | 871.4686 | 871.4683 | −0.34 | 871.4683 (3.49), 739.4295 (0.26), 577.3759 (3.89), 415.3226 (20.38), 397.3119 (7.62), 379.3015 (0.85), 273.2224 (58.89), 255.2119 (100.00) | MS/MS |
| 68  | 45.85           | 25(S)-Schidigera-saponin D\(_4\) | C45H72O18 | [M + H]\(^+\) | 903.4948 | 903.4932 | −1.77 | 903.4932 (0.26), 741.4412 (6.04), 579.3887 (100.00), 417.3372 (39.49), 399.3286 (10.50), 273.2224 (37.44), 255.2121 (41.63) | MS/MS, literature [5] |
| 69  | 46.25           | 5β-Spirost-25(27)-en-3\(\beta\),12\(\beta\)-diol \# | C27H42O4 | [M + H]\(^+\) | 431.3156 | 431.3175 | 4.41 | 431.3175 (77.53), 413.3070 (10.73), 395.2965 (11.49), 289.2169 (70.91), 283.2458 (100.00), 271.2064 (13.97), 253.1960 (11.87) | MS/MS |
| 70  | 46.40           | 25(RS)-5β-Spirostan-2β,3β-diol 3-O-Xyl(1→3)-Gal \# | C38H52O13 | [M + H]\(^+\) | 727.4263 | 727.4297 | 4.67 | 727.4297 (2.08), 595.3818 (3.68), 577.3726 (3.81), 433.3324 (52.35), 415.3224 (98.24), 397.3102 (7.81), 301.2544 (10.27), 289.2170 (91.96), 271.2063 (100.00), 253.1959 (41.68) | MS/MS |
| No. | \( t_R \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z (Intensity)) | Identification Method |
|-----|-----------------|----------|---------|-------------|-----------|--------|-----|------------------------|----------------------|
| 71  | 46.55           | Ys-IV    | \( \text{C}_{43}\text{H}_{74}\text{O}_{18} \) | [M + H]\(^+\) | 903.4948  | 903.4932 | −1.77 | 903.4932 (0.26), 741.4412 (6.04), 579.3928 (3.99) | MS/MS, literature [5] |
| 72  | 46.88           | Schidigera-saponin A\(^1\) * | \( \text{C}_{44}\text{H}_{71}\text{O}_{17} \) | [M + H]\(^+\) | 871.4686 | 871.4693 | 0.80 | 871.4693 (0.94), 739.4294 (0.07), 577.3754 (5.44), 415.3222 (22.22), 397.3116 (9.62), 379.3011 (0.29) | MS/MS, standard reported by literature [1] |
| 73  | 47.26           | 25(S)-Schidegera-saponin D\(_2\) | \( \text{C}_{44}\text{H}_{72}\text{O}_{17} \) | [M + H]\(^+\) | 873.4842 | 873.4800 | −4.81 | 873.4800 (0.32), 741.4323 (0.61), 579.3882 (7.97), 417.3358 (35.70), 399.3250 (15.86), 381.3137 (2.33), 273.2207 (35.79), 255.2104 (100.00) | MS/MS, literature [5] |
| 74  | 48.01           | 25(R)-Schidegera-saponin D\(_2\) | \( \text{C}_{44}\text{H}_{72}\text{O}_{17} \) | [M + H]\(^+\) | 873.4842 | 873.4879 | 4.24 | 873.4879 (0.32), 741.4323 (0.61), 579.3882 (7.97), 417.3358 (35.70), 399.3250 (15.86), 381.3137 (2.33), 273.2207 (35.79), 255.2104 (100.00) | MS/MS, literature [5] |
| 75  | 48.22           | 25(S)-Schidigera-saponin D\(_3\) * | \( \text{C}_{45}\text{H}_{74}\text{O}_{18} \) | [M + H]\(^+\) | 903.4948 | 903.4937 | −1.22 | 903.4937 (2.00), 722.1501 (1.73), 579.3891 (3.38), 417.3375 (25.17), 399.3263 (7.83), 273.2226 (27.94), 255.2118 (100.00) | MS/MS, standard isolated by our lab |
| 76  | 48.49           | Mexogenin | \( \text{C}_{27}\text{H}_{42}\text{O}_{5} \) | [M + H]\(^+\) | 447.3105 | 447.3113 | 1.79 | 447.3113 (32.13), 429.3023 (100.00), 411.2913 (19.45), 315.2332 (9.37), 285.1844 (1.12), 253.1693 (1.93) | MS/MS, literature [5] |
| 77  | 48.82           | Timosaponin A III | \( \text{C}_{39}\text{H}_{64}\text{O}_{13} \) | [M + H]\(^+\) | 741.4420 | 741.4452 | 4.32 | 741.4452 (2.37), 579.3932 (3.64), 417.3382 (10.01), 399.3280 (9.12), 285.2587 (3.32), 273.2222 (39.02), 255.2117 (100.00) | MS/MS, literature [14] |
| 78  | 48.99           | Ys-III | \( \text{C}_{45}\text{H}_{74}\text{O}_{18} \) | [M + H]\(^+\) | 903.4948 | 903.4937 | −1.22 | 903.4937 (2.00), 722.1495 (0.91), 579.3891 (3.59), 417.3371 (27.58), 399.3271 (9.06), 273.2222 (30.82), 255.2118 (100.00) | MS/MS, literature [5] |
| 79  | 49.49           | Asparinin A | \( \text{C}_{39}\text{H}_{64}\text{O}_{13} \) | [M + H]\(^+\) | 741.4420 | 741.4432 | 1.62 | 741.4432 (1.50), 579.3926 (4.93), 417.3386 (10.53), 399.3273 (8.40), 285.2581 (3.77), 273.2222 (39.90), 255.2118 (100.00) | MS/MS, literature [15] |
| 80  | 50.00           | Tuberosine A | \( \text{C}_{33}\text{H}_{64}\text{O}_{9} \) | [M + H]\(^+\) | 595.3841 | 595.3868 | 4.53 | 595.3868 (24.29), 433.3332 (23.11), 415.3225 (56.28), 397.3112 (17.73), 301.2546 (6.96), 289.2172 (75.61), 283.2436 (103.33), 271.2067 (100.00), 253.1959 (52.17) | MS/MS, literature [16] |
| No. | $r_g$ (min) | Compound | Formula | Adduct Ions | Theoretic Measure | Diff | MS/MS (m/z (Intensity)) | Identification Method |
|-----|-------------|----------|---------|-------------|-------------------|------|------------------------|----------------------|
| 82  | 50.01       | 5β-Spirost-25(27)-en-3β-yl 3-O-Glc(1→2)-Glc § | C$_{39}$H$_{58}$O$_{17}$ | [M + H]$^+$ | 739.4263          | 2.57 | 739.4282 (1.09), 577.3763 (2.43), 415.3220 (4.80), 397.3107 (6.67), 379.3027 (1.41), 285.2587 (2.73), 273.2221 (30.31), 255.2116 (100.00) | MS/MS                |
| 83  | 50.76       | 25(S)-Schidigera-saponin D$_5$ * | C$_{39}$H$_{64}$O$_{13}$ | [M + H]$^+$ | 741.4420          | 3.51 | 741.4446 (4.25), 579.3954 (0.74), 417.3398 (6.71), 399.3262 (2.94), 285.2582 (1.86), 273.2223 (29.87), 255.2118 (100.00) | MS/MS, standard reported by literature [6] |
| 84  | 51.46       | 25(S)-Schidigera-saponin D$_1$ * | C$_{44}$H$_{72}$O$_{17}$ | [M + H]$^+$ | 873.4842          | 3.89 | 873.4876 (0.32), 741.4233 (0.61), 579.3867 (6.82), 417.3355 (35.08), 399.3251 (14.24), 381.3162 (2.60), 273.2209 (32.22), 255.2104 (100.00) | MS/MS, standard reported by literature [6] |
| 85  | 51.65       | 25(R)-Schidigera-saponin D$_5$ * | C$_{39}$H$_{64}$O$_{13}$ | [M + H]$^+$ | 741.4420          | 1.89 | 741.4443 (2.52), 579.3890 (0.90), 417.3380 (7.65), 399.3269 (5.68), 285.2586 (2.06), 273.2211 (30.08), 255.2117 (100.00) | MS/MS, standard reported by literature [6] |
| 86  | 52.10       | 25(R)-Schidigera-saponin D$_1$ * | C$_{44}$H$_{72}$O$_{17}$ | [M + H]$^+$ | 873.4842          | 3.89 | 873.4876 (0.32), 741.4232 (0.61), 579.3867 (6.82), 417.3355 (35.08), 399.3251 (14.24), 381.3162 (2.60), 273.2209 (32.22), 255.2104 (100.00) | MS/MS, standard reported by literature [6] |
| 87  | 53.62       | Schidegeragenin B | C$_{27}$H$_{48}$O$_{3}$ | [M + H]$^+$ | 429.2999           | 3.73 | 429.3015 (100.00), 411.2890 (43.35), 393.2812 (36.09), 299.2374 (19.35), 281.2256 (19.25), 269.1908 (8.16) | MS/MS, literature [5] |
| 88  | 53.66       | (25S)-5β-Spirostan-3β-yl 3-O-Glc(1→3)-Gal § | C$_{39}$H$_{64}$O$_{13}$ | [M + H]$^+$ | 741.4420          | 2.97 | 741.4442 (0.53), 417.3377 (3.74), 399.3259 (2.41), 273.2216 (34.83), 255.2114 (100.00) | MS/MS |
| 89  | 53.99       | (25R/S)-12β-Hydroxysmilagenin | C$_{27}$H$_{44}$O$_{4}$ | [M + H]$^+$ | 433.3312           | 2.08 | 433.3321 (100.00), 289.2173 (21.95), 283.2426 (22.32), 271.2060 (9.32), 253.1963 (10.37) | MS/MS, literature [10] |
| 90  | 54.20       | 5β-Spirost-25(27)-en-3β-ol 3-O-Xyl(1→3)-Gal § | C$_{39}$H$_{60}$O$_{12}$ | [M + H]$^+$ | 709.4158           | 1.27 | 709.4167 (12.74), 567.3175 (1.32), 435.2757 (1.81), 415.3192 (6.10), 397.3103 (4.17), 273.2222 (23.06), 255.2116 (100.00) | MS/MS |
| 91  | 54.31       | (25R)-5β-Spirostan-3β-yl 3-O-Glc(1→3)-Gal § | C$_{39}$H$_{64}$O$_{13}$ | [M + H]$^+$ | 741.4420          | 4.32 | 741.4452 (2.12), 579.3917 (1.41), 417.3338 (8.83), 399.3278 (7.04), 285.2578 (2.34), 273.2221 (31.71), 255.2117 (100.00) | MS/MS |
| No. | \( t_R \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z/Intensity) | Identification Method |
|-----|----------------|------------------|----------|-------------|-----------|---------|-----|----------------------|----------------------|
| 92  | 55.22 | 5β-Spirost-25(27)-en-3β-yl 3-O-Glc(1→3)-Glc \* | \( \text{C}_{39}\text{H}_{42}\text{O}_{13} \) | \([M + H]^+\) | 739.4263 | 739.4254 | −0.12 | 739.4254 (7.51), 577.3735 (1.71), 415.3225 (6.43), 397.3108 (8.13), 379.3026 (1.03), 285.2591 (2.67), 273.2221 (33.26), 255.2117 (100.00) | MS/MS |
| 93  | 55.22 | 25(RS)-5β-Spirost-3β-ol 3-O-Gal | \( \text{C}_{33}\text{H}_{14}\text{O}_{6} \) | \([M + H]^+\) | 579.3891 | 579.3913 | 0.80 | 579.3913 (14.18), 435.2762 (11.61), 399.3256 (0.97), 285.2598 (0.93), 273.2221 (14.70), 255.2116 (100.00) | MS/MS, literature [17] |
| 94  | 55.32 | 25(S)-5β-Spirost-3β-ol 3-O-Xyl(1→3)-Gal \* | \( \text{C}_{38}\text{H}_{42}\text{O}_{12} \) | \([M + H]^+\) | 711.4314 | 711.4335 | −2.05 | 711.4335 (7.88), 567.3163 (22.2), 435.2789 (2.10), 417.3373 (6.00), 399.3256 (6.41), 285.2584 (4.27), 273.2221 (29.40), 255.2114 (100.00) | MS/MS |
| 95  | 55.51 | (25S)-5β-Spirost-3β-ol-12-one | \( \text{C}_{27}\text{H}_{42}\text{O}_{4} \) | \([M + H]^+\) | 431.3156 | 431.3177 | −4.87 | 431.3177 (100.00), 413.3067 (46.79), 395.2965 (25.11), 377.2840 (0.25), 317.2492 (9.30), 299.2379 (39.06), 281.2274 (24.81) | MS/MS, literature [5] |
| 96  | 56.04 | 25(R)-5β-Spirost-3β-ol 3-O-Xyl(1→3)-Gal \* | \( \text{C}_{38}\text{H}_{42}\text{O}_{12} \) | \([M + H]^+\) | 711.4314 | 711.4305 | −1.27 | 711.4305 (6.51), 579.3914 (1.57), 417.3357 (3.88), 399.3264 (4.26), 285.2581 (2.83), 273.2220 (26.63), 255.2116 (100.00) | MS/MS |
| 97  | 56.37 | Yucca spirostanoside A2 | \( \text{C}_{38}\text{H}_{40}\text{O}_{12} \) | \([M + H]^+\) | 709.4158 | 709.4163 | 0.70 | 709.4163 (4.36), 577.3773 (1.06), 415.3223 (3.53), 397.3117 (4.25), 379.3006 (0.11), 285.2590 (1.71), 273.2219 (26.74), 255.2116 (100.00) | MS/MS, standard replot by literature [1] |
| 98  | 56.43 | 25(S)-5β-Spirost-3β-ol 3-O-Glc(1→3)-Glc \* | \( \text{C}_{39}\text{H}_{44}\text{O}_{13} \) | \([M + H]^+\) | 741.4420 | 741.4414 | −0.16 | 741.4414 (1.19), 579.3919 (3.14), 417.3380 (11.15), 399.3275 (14.43), 285.2584 (4.44), 273.2223 (43.87), 255.2118 (100.00) | MS/MS |
| 99  | 57.03 | 25(R)-5β-spirost-3β-ol 3-O-Glc(1→3)-Glc \* | \( \text{C}_{39}\text{H}_{44}\text{O}_{13} \) | \([M + H]^+\) | 741.4420 | 741.4436 | 2.16 | 741.4436 (1.11), 579.3913 (3.32), 417.3377 (11.40), 399.3276 (14.08), 285.2584 (4.09), 273.2223 (40.84), 285.2579 (1.15), 255.2118 (100.00) | MS/MS |
| 100 | 57.13 | Yucca spirostanoside A1 | \( \text{C}_{39}\text{H}_{44}\text{O}_{13} \) | \([M + H]^+\) | 577.3735 | 577.3737 | 0.35 | 577.3737 (10.11), 415.3222 (20.05), 397.3145 (0.89), 379.3027 (0.60), 285.2592 (17.54), 255.2117 (100.00) | MS/MS, standard replot by literature [1] |
| 101 | 57.79 | (25R)-Spirostan-3β-ol-12-one | \( \text{C}_{27}\text{H}_{42}\text{O}_{4} \) | \([M + H]^+\) | 431.3156 | 431.3162 | 1.39 | 431.3162 (100.00), 413.3064 (37.68), 395.2967 (23.34), 377.2850 (5.94), 317.2499 (2.84), 299.2378 (25.93), 281.2275 (23.20) | MS/MS, literature [5] |
| 102 | 57.99 | 25(S)-5β-Spirost-3β-ol 3-O-Xyl(1→3)-Glc \* | \( \text{C}_{38}\text{H}_{42}\text{O}_{12} \) | \([M + H]^+\) | 711.4314 | 711.4305 | −1.27 | 711.4305 (5.95), 579.3902 (1.23), 417.3376 (7.78), 399.3273 (9.77), 285.2580 (3.40), 273.2221 (33.53), 255.2115 (100.00) | MS/MS |
Table 1. Cont.

| No. | \( r_k \) (min) | Compound | Formula | Adduct Ions | Theoretic | Measure | Diff | MS/MS (m/z) (Intensity) | Identification Method |
|-----|----------------|----------|---------|-------------|-----------|--------|-----|------------------------|-----------------------|
| 103 | 58.02          | 25(R)-5β-Spirostan-3β-ol 3-O-Xyl(1→3)-Glc \(^*\) | C\(_{36}\)H\(_{42}\)O\(_{12}\) | [M + H]\(^+\) | 711.4314 | 711.4323 | 1.27 | 711.4323 (1.77), 579.3886 (0.76), 417.3372 (6.68), 399.3280 (8.46), 285.2591 (3.66), 273.2224 (33.80), 255.2119 (100.00) | MS/MS |
| 104 | 58.57          | (25R)-5β-Spirostan-3β-ol 3-O-Glc \(^*\) | C\(_{33}\)H\(_{44}\)O\(_{8}\) | [M + H]\(^+\) | 579.3891 | 579.3918 | 4.66 | 579.3918 (16.27), 435.2755 (11.79), 417.3367 (0.02), 399.3271 (1.97), 381.3169 (0.03), 285.2588 (11.11), 273.2220 (17.83), 255.2118 (100.00) | MS/MS, standard reported by literature [6] |
| 105 | 58.62          | Asparagoside A \(^*\) | C\(_{33}\)H\(_{44}\)O\(_{8}\) | [M + H]\(^+\) | 579.3891 | 579.3911 | 3.45 | 579.3911 (16.39), 435.2753 (12.48), 417.3367 (0.02), 399.3271 (1.97), 381.3169 (0.03), 285.2588 (11.11), 273.2220 (17.92), 255.2117 (100.00) | MS/MS, standard reported by literature [6] |
| 106 | 58.72          | Samogenin/Markogenin | C\(_{27}\)H\(_{44}\)O\(_{4}\) | [M + H]\(^+\) | 433.3312 | 433.3330 | 4.15 | 433.3330 (53.36), 415.3216 (5.59), 301.2562 (1.14), 289.2174 (100.00), 283.2453 (12.11), 271.2068 (56.56), 253.1962 (33.89) | MS/MS, literature [5] |
| 107 | 60.36          | (25S)-Isohodeasapogenin | C\(_{27}\)H\(_{44}\)O\(_{4}\) | [M + H]\(^+\) | 433.3312 | 433.3324 | 2.77 | 433.3324 (19.22), 415.3218 (10.46), 391.2530 (4.85), 289.2169 (5.66), 283.2421 (17.61), 271.2064 (17.78), 253.1961 (28.29) | MS/MS, literature [13] |
| 108 | 61.29          | Schidigeragenin A\(_1\) | C\(_{27}\)H\(_{42}\)O\(_{3}\) | [M + H]\(^+\) | 415.3207 | 415.3225 | 4.33 | 415.3225 (85.22), 397.3115 (18.74), 379.3015 (8.75), 285.2586(2.26), 273.2224 (100.00), 255.2119 (86.40) | MS/MS, literature [5] |
| 109 | 63.73          | Sarsasapogenin | C\(_{27}\)H\(_{44}\)O\(_{3}\) | [M + H]\(^+\) | 417.3363 | 417.3382 | 4.55 | 417.3382 (35.52), 285.2589 (1.44), 273.2226 (100.00), 255.2120 (70.59) | MS/MS, literature [5] |
| 110 | 64.08          | Smilagenin | C\(_{27}\)H\(_{44}\)O\(_{3}\) | [M + H]\(^+\) | 417.3363 | 417.3348 | −3.59 | 417.3348 (35.68), 285.2599 (1.38), 273.2226 (100.00), 255.2117 (72.83) | MS/MS, literature [5] |

\(^*\) The compounds unambiguously identified with the reference standards comparison; \(^\#\) The potential new compounds; The underline annotation indicated that the structures of compounds were proved by targeted isolation.
2.2.2. Rationale for Structural Characterization

According to the references [1,6,10,12,18], the spirostanol saponins from YS were composed by ten kinds of spirostanol aglycons (YS-1–YS-10, Figure 2) linked with nine kinds of glycosyl groups (A1–A9, Figure 2, Table S8) which was made up by glucopyranosyl (Glc), galactopyranosyl (Gal) and xylopyranosyl (Xyl) at C-3 of the aglycons. Among them, YS-7, YS-9 and YS-10 were only mentioned in references and haven’t been obtained by our lab. The occurrence probability of these three kinds of glycosyl groups was Glc > Gal/Xyl.

When YS-1–YS-3 and YS-6–YS-8 were glycosylated, both Glc and Gal could be directly linked to the C-3 of aglycon, and Glc substitute was more likely to occur, while YS-4, YS-5, YS-9, YS-10 (hydroxyl substituted at C-2) were prone to be glycosylated by Gal directly at C-3 [5,18].

At the same time, the glycosyls directly linked with aglycons (DG) were limited to Glc and Gal, and whose 2', 3' and/or 6'-positions were easily substituted by other kinds of glycosyls, and the characteristic types are listed in Table 1. When YS-1–YS-3 and YS-6–YS-8 were glycosylated, both Glc and Gal could be directly linked to the C-3 of aglycon, and Glc substitute was more likely to occur, while YS-4, YS-5, YS-9, YS-10 (hydroxyl substituted at C-2) were prone to be glycosylated by Gal directly at C-3 [5,18].

Figure 2. Structures of ten aglycons and nine glycosyls of spirostanol saponins from YS.

Study on MS/MS Cleavage Pattern of Spirostanol Saponins from YSESs

According to what we have summarized, the aglycons of the spirostanol saponins from YSESs were usually substituted by -OH, thus dehydration reactions often happened, and a series of neutral 18 Da losses were produced. Moreover, the E-ring cleavage of $\Delta^{25(27)}$ spirostanol saponins was prone to lose 112 Da, forming a $[M-C_6H_5O_2 + H]^+$ fragment ion, or lose 142 Da to form a $[M-C_8H_{14}O_2 + H]^+$ fragment ion, while the 25-CH$_3$ spirostanol saponins could lose 114 Da to yield a $[M-C_8H_{10}O_2 + H]^+$ fragment ion, or 144 Da was lost, forming a $[M-C_8H_{18}O_2 + H]^+$ fragment ion. The different $m/z$ as well as the different kinds of cleavage fragment ions (Figure 3, Table S9) could be used to quickly identify the different types of spirostanol saponins from YSESs. The two pairs of aglycons YS-2 and YS-4 which exhibited the same molecular and fragment ion composition, could be distinguished by observing the relative intensity of the $m/z$ 283.2420 ion. In detail, because of the 12-OH substitution of YS-2, in its MS/MS spectrum, the intensity of $m/z$ 283.2420 ion was stronger than that of $m/z$ 289.2162, while it was opposite of what was observed in the spectrum of 12-H$_2$ substituted YS-4. This phenomenon has been observed in the spectrum of YS-9 as well. Although a sample possessing the YS-7 aglycon was lacking, we designed a targeted separation, in order to verify the above rule.
Figure 3. The MS/MS spectrum and proposed fragmentation pathways of YS‑1–YS‑6 and YS‑8.
Study on MS/MS Cleavage Pattern of Spirostanol Saponins from YSES

By comparing the chromatographic elution order of reference standards (peaks 16, 17, 31, 32, 35, 40, 41, 51, 52, 60, 66, 83–86, 100, 104), it was found that the retention times of this class of compounds were mainly affected by the substitution type of the C-12 and C-25 of the aglycon, the stereochemistry of 25-CH₃, as well as the type of substituted glycosyl (Table S10).

2.2.3. Structural Elucidation of Spirostanol Saponins from YSES

Using the rules summarized above, another seventy-nine spirotanol saponins have been tentatively identified. In this section, the tentative identification of ten novel 25(R/S)-spirotanol saponins would be described in detail. What’s more, the speculation would be verified by their subsequent targeted isolation.

In the MS/MS spectra of peak 10 (m/z 903.4550), characteristic ions at m/z 447.3109, 411.2903, 393.2797, 333.2435, 315.2324, 285.1842 could be observed (Table 1, Figure S8). The relative molecular mass of its aglycon was 2 Da more than that of YS-5. The ions of m/z 333.2345, 315.2324, 285.1842 formed by the cleavage of E ring were similar to those of YS-5 as well, which indicated that the A–E ring was the same as in YS-5. As a result, the aglycon of peak 10 was tentatively assumed to be 25(R/S)-5β-spirostan-2β,3β-ol-12-one spirotanol. It was speculated that its glycosyl substituent consisted of two molecules of hexose and one pentose molecule because of its neutral loss of 294 Da and 162 Da. According to the biosynthetic pathway of Y. schidigera, the structure of peak 10 could be tentatively speculated to be either 25(R/S)-5β-spirostan-2β,3β-ol-12-one-3-O-xylopyranosyl(1→3)-[glucopyranosyl(1→2)]-glucopyranoside or 25(R/S)-5β-spirostan-2β,3β-ol-12-one-3-O-xylo-pyranosyl (1→3)-[glucopyranosyl(1→2)]-galactopyranoside. In order to clarify the correctness of above speculation, a targeted separation was conducted, and peak 10 was finally unambiguously identified as a novel compound, named as 25(R)-5β-spirostan-2β,3β-ol-12-one-3-O-xylopyranosyl(1→3)-[glucopyranosyl(1→2)]-galactopyranoside.

In the MS/MS spectrum of peaks 14 (m/z 889.4772), 15 (m/z 889.4772), 34 (m/z 727.4261), 36 (m/z 727.4261), 40 (m/z 595.3868), and 41 (m/z 595.3866), characteristic ions at m/z 433.3321, 397.3101, 379.2995, 301.2526, 289.2162, 283.2420, 271.2056, 253.1951 could be observed (Table 1, Figures S9, S10, S12, S13, S15 and S16). According to what we have summarized in the Section “Study on MS/MS Cleavage Pattern of Spirostanol Saponins from YSES”, as the intensities of all their m/z 283.2420 peaks were stronger than those of the m/z 289.2162 ones, their aglycon was proposed to be the 12-OH isomer of YS-9, and in particular, it might be YS-7. According to their neutral ion losses, peak 14 [m/z 889.4772, 595.3867 (294 Da loss) and 433.3323 (162 Da loss)], peak 15 [m/z 889.4767, 595.3850 (294 Da loss) and 433.3330 (162 Da loss)], peak 34 [m/z 727.4261, 595.3719 (152 Da loss) and 433.3330 (162 Da loss)], peak 36 [m/z 727.4261 and 433.3314 (294 Da loss)], peak 40 [m/z 595.3864 and 433.2562 (162 Da loss)], peak 41 [m/z 595.3864 and 433.2563 (162 Da loss)] were indicated to be substituted by two hexoses and one pentose, two hexoses and one pentose, one hexose and one pentose, one hexose and one norchexose and one pentose, one hexose and one norchexose, respectively. The MS/MS spectra of peaks 14 and 15, 34 and 36, 40 and 41 were almost identical, and their retention times were also very close, therefore, they were conjectured to be three pairs of (25R/S)-isomers with the YS-7 aglycon. Considering the biosynthetic pathway of Y. schidigera as well as the chromatographic elution order, peaks 14, 15, 34, 36, 40 and 41 were speculated to be 25(S)-5β-spirostan-3β,12β-diol 3-O-xylopyranosyl(1→3)-[glucopyranosyl(1→2)]-glucopyranoside (14), 25(R)-5β-spirostan-3β,12β-diol 3-O-xylopyranosyl(1→3)-[glucopyranosyl(1→2)]-glucopyranoside (15), 25(S)-5β-spirostan-3β,12β-diol 3-O-xylopyranosyl(1→3)-glucopyranoside (34), 25(R)-5β-spirostan-3β,12β-diol 3-O-xylopyranosyl(1→3)-glucopyranoside (36), 25(S)-5β-spirostan-3β,12β-diol 3-O-glucopyranoside (40) and 25(R)-5β-spirostan-3β,12β-diol 3-O-glucopyranoside (41), respectively. Furthermore, they were all novel compounds, and targeted for separation to verify the above speculation.

For peaks 27 (m/z 887.4648), 38 (m/z 887.4665), 51 (m/z 755.4223), characteristic ions at m/z 431.3156, 413.3050, 395.2945, 377.2839, 299.2369, 281.2264, 269.1900 could be observed in their MS/MS spectra.
were consistent with those of yucca spirostanoside D. The neutral losses of peaks 27 [m/z 887.4518, 755.4220 (132 Da loss), 593.3700 (162 Da loss) and 431.3167 (162 Da loss)], peak 38 [m/z 887.4665, 593.3695 (294 Da loss) and 431.3172 (162 Da loss)], and peak 51 [m/z 755.4230, 593.3699 (162 Da loss) and 431.3173 (162 Da loss)] suggested they were substituted by two hexose molecules and one pentose molecule, two hexose molecules and one pentose molecule and two hexose molecules, respectively. By referring to references as well as comparing them to reference standards, peaks 24 and 32 were the isomers of peaks 27 and 38, which have been identified to be 25(R)-5β-spirostan-3β-ol-12-one 3-O-xylopyranosyl(1→3)[glucopyranosyl(1→2)]-galactopyranoside (24) and 25(R)-5β-spirostan-3β-ol-12-one 3-O-xylopyranosyl(1→3)[glucopyranosyl(1→2)]-glucopyranoside (32), respectively. Thus peaks 27 and 38 were tentatively speculated to be YS-8 substituted by two hexose molecules and one pentose molecule. During the targeted separation and structure elucidation, they were unambiguously identified as 25(R)-5β-spirostan-3β-ol-12-one 3-O-apiofuranosyl(1→3)-[glucopyranosyl(1→2)]-glucopyranoside (27) and 25(R)-5β-spirostan-3β-ol-12-one 3-O-xylopyranosyl(1→3)-[glucopyranosyl(1→2)]-glucopyranoside (38), respectively. Similarly, peak 51 was unambiguously identified as 25(R)-5β-spirostan-3β-ol-12-one 3-O-glucopyranosyl (1→5)-glucopyranoside (51). Peaks 27, 38 and 51 are all novel compounds.

Similar MS/MS cleavage patterns and chromatographic elution orders as well as the comparisons with references (Table 1) were used to identify another seventy-nine spirostanol saponins, among which, thirty of them were tentatively new.

2.2.4. Targeted Separation of Peaks 10, 14, 15, 27, 34, 36, 38, 40, 41, 51

For the purpose of verifying the above speculations, targeted separation of peaks 10, 14, 15, 27, 34, 36, 38, 40, 41, 51 was carried out. In the light of the MPLC-MS analysis results, peak 10 was found to be enriched in the “Preparation of the YSESs Test Solutions” fraction 9; peaks 14, 15 and 38 were found to be enriched in the “Preparation of the YSESs Test Solutions” fraction 8; peak 27 was found to be enriched in the “Preparation of the YSESs Test Solutions” fraction 7; peaks 34, 36, 40, 41 and 51 were found to be enriched in the “Preparation of the YSESs Test Solutions” fraction 6. Then silica gel, ODS CC and preparative HPLC (pHPLC) were used to isolate these fractions and the spirostanol saponins 10, 14/15, 27, 34/36, 38, 40/41, 51 were thus obtained.

(25R)-5β-Spirostan-2β,3β-diol-12-one 3-O-β-D-xylopyranosyl(1→3)-[β-D-glucopyranosyl(1→2)]-β-D-galactopyranoside (10)

A white powder with negative optical rotation ([α]_D^25 ~ -7.7, MeOH). Its molecular formula, C_{44}H_{59}O_{19}, was deduced by positive-ion ESI-Q-Exactive-Orbitrap MS (m/z 903.4550 [M+H]^+; calc for C_{44}H_{59}O_{19} 903.4584). Acid hydrolysis yielded β-galactose, β-glucose, and β-xylose [1]. The 1H- and 13C-NMR (Table S1) spectra suggested the presence of one β-D-galactopyranosyl ([δ 4.98 (1H, d, J = 7.5 Hz, H-1’)], one β-D-glucopyranosyl ([δ 5.57 (1H, d, J = 7.5 Hz, H-1’)], together with one β-D-xylopyranosyl ([δ 5.23 (1H, d, J = 8.0 Hz, H-1’)]). The 13C-NMR signals attributed to the sugar moieties of 10 were consistent with those of yucca spirostanoside D_{1} (5β-spirost-25(27)-en-2β,3β-diol-12-one 3-O-β-D-xylopyranosyl(1→3)-[β-D-glucopyranosyl(1→2)]-β-D-galactopyranoside) [1]. A combination of HSQC, HSQC-TOCSY, and 1H 1H COSY spectra analysis led to the assignment of the three glycosyl units. Forty-four carbon signals have been observed in its 13C-NMR spectrum. In addition to the carbon signals represented by the above three glycosyl units, the other 27 signals indicated 10 was a spirostane-type steroid saponin. Its 1H- and 13C-NMR spectra showed signals for two tertiary methyl groups at δ_{H} 0.98, 1.08 (3H each, both s, H-3′, 18), one secondary methyl group at δ_{H} 1.35 (3H, d, J = 7.0 Hz, H-21), one oxygenated methylene group at δ_{H} 3.50 (1H, dd, J = 10.5, 10.5 Hz), 3.59 (1H, m, overlapped), H-26], three oxygenated methine protons at δ_{H} 3.75, 4.40 (1H each, both m, overlapped, H-2, 3), 4.53 (1H, q like, ca. J = 9 Hz, H-16)], together with one carboxyl carbon at δ_{C} 212.7 (C-12). The 1H 1H COSY spectrum of 10 suggested the presence of the three partial structures indicated by bold lines in Figure 4. The planar structure of the aglycon was determined based on the key HMBC correlations from H-3′, 18 to C-12–14, C-17; H-3′, 19 to C-1, C-5, C-9, C-10; H-25 to C-17, C-20, C-22; H-26.
to C-22; H2-27 to C-24-26. Moreover, the connection positions of glycosyl units were determined by the long-range correlations from H-1' to C-3, H-1'' to C-2', H-1''' to C-3' observed in the HMBC experiment. The 1H- and 13C-NMR data for the protons and carbons in the A–E rings were identical to those of Yucca spirostanoside D3 [1], thus the configuration of the A–E rings was determined. The comparison results of its 13C-NMR data for F ring (C-22–26) and C-27 [δ 17.3 (C-27), 29.2 (C-24), 30.5 (C-25), 31.8 (C-23), 67.0 (C-26), 109.3 (C-22)] with those of (25R)-5-spirostan [817.3 (C-27), 29.2 (C-24), 30.5 (C-25), 31.8 (C-23), 67.0 (C-26), 109.3 (C-22)] [11], further clarified the absolute configuration of C-25. As a result, the structure of 10 was identified as (25R)-5β-spirostan-2β,3β-diol-12-one 3-O-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→2)β-D-galactopyranoside.

Figure 4. The main 1H-1H COSY and HMBC correlations of 10, 14/15, 27, 34/36, 38, 40/41, 51.

25(S/R)-5β-spirostan-3β,12β-diol 3-O-β-D-xylopyranosyl(1→3)-[β-D-glucopyranosyl(1→2)]
-β-D-galactopyranoside (14/15), 25(S/R)-5β-spirostan-3β,12β-diol
3-O-β-D-xylopyranosyl(1→3)-β-D-gluco-pyranoside (34/36) and 25(S/R)-5β-spirostan-3β,12β-diol
3-O-β-D-gluco-pyranoside (40/41)

The 1H- and 13C-NMR (Tables S2, S4 and S6, respectively) spectra of 14/15, 34/36 and 40/41 indicated that the three pairs of spirostanol saponins possessed the same aglycon, which was very similar to that of 10, 25(R/S)-5β-spirostan-3β,12β-diol. The difference was that the 2-OH was lacking in the structures of 14/15, 34/36 and 40/41, meanwhile the 12-C=O of 10 has been changed into a 12-OH group. The long-range correlations from H-14, 17 and H2-18 to C-12 observed in their HMBC spectra as well as the correlations between H2-2 and H-3 displayed in their 1H-1H COSY spectra verified the correctness of the above speculation. By comparing the C-22–26 and 27 carbon signals of 10, 14/15, 34/36 and 40/41 they were determined to be 25R and 25S isomer mixtures. As their 1H- and 13C-NMR data were consistent with those of 25(R and S) schidigera-saponin F1 [6], their aglycon was identified as 25(R/S)-5β-spirostan-3β,12β-diol. Acid hydrolysis yielded D-galactose, D-glucose, and D-xylose; D-glucose and D-xylose; and D-glucose,
were identified by the long-range correlations from H-1. Acid hydrolysis only yielded 34/36 possessed one β-β-glucopyranosyl (δ 4.90 (1H, d, δJ = 8.0 Hz, H-1′)) [4], together with one β-β-xylopyranosyl (δ 5.29 (1H, d, δJ = 7.5 Hz, H-1′′)). 34/36 possessed one β-β-glucopyranosyl (δ 4.90 (1H, d, δJ = 8.0 Hz, H-1′)) [4] and one β-β-xylopyranosyl (δ 5.27 (1H, d, δJ = 7.5 Hz, H-1′′)). Finally, combining the long-range correlations from H-1′ to C-3, H-1′′ to C-2′, and H-1′′′ to C-3′ for 34/36, H-1′ to C-3 for 34/36 and 25(S)-5β-spirostan-3β,12β-diol 3-O-β-β-glucopyranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-β-d-glucopyranoside (34/36) and 25(S)-5β-spirostan-3β,12β-diol 3-O-β-β-glucopyranoside (40/41), respectively. According to the chromatographic elution order summarized in Table S10, the retention time of 25(S)-spiropstanol saponin was shorter than that of 25(R)-spiropstanol saponin, thus the six peaks were finally identified as 25(S)-5β-spirostan-3β,12β-diol 3-O-β-β-xylopyranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-β-glucopyranoside (14), 25(R)-5β-spirostan-3β,12β-diol 3-O-β-β-xylopyranosyl (1→3)-[β-β-glucopyranosyl(1→2)]-β-β-d-glucopyranoside (15), 25(S)-5β-spirostan-3β,12β-diol 3-O-β-β-xylopyranosyl(1→3)-β-β-d-glucopyranoside (34), 25(R)-5β-spirostan-3β,12β-diol 3-O-β-β-xylopyranosyl (1→3)-β-β-d-glucopyranoside (36), 25(S)-5β-spirostan-3β,12β-diol 3-O-β-β-d-glucopyranoside (40) and 25(R)-5β-spirostan-3β,12β-diol 3-O-β-β-glucopyranoside (41), respectively.

Compounds 27, 38 and 51 were all white powders with negative optical rotations. They were determined to possess the same aglycon by comparing their 1H- and 13C-NMR (Tables S5, S7 and S9, C5D5N, C5D5N data with each other, that was identical to the speculation in Section 2.2.3. The 1H-, 13C-NMR and 2D-NMR spectra (1H 1H COSY, HSQC, HMBC) spectra of the aglycon indicated that it was similar to that of 14/15, the difference being that the 12-OH group was lacking while a 12-C-O appeared in the structures of these three compounds. This speculation was proven by the long-range correlations from H-14, H-17 and H-18 to C-12 observed in their HMBC spectra (Figure 4). In addition, the pentose signals of 27 and 38 were changed as well. According to the literature, the pentose in the structures of 27 and 38 was elucidated to be β-β-apiofuranosyl [19]. The HSQC, and HSQC-TOCSY combined with COSY spectra were used for the assignment of glycosyl units. Their connections were identified by the long-range correlations from H-1′ to C-3, H-1’′ to C-2′, H-1’’′ to C-3′ observed in their HMBC spectra. For 51, there were thirty-nine carbon signals in the 13C-NMR spectrum. Aside from the twenty-seven carbons belonging to the aglycon, twelve carbons were left to assign. Acid hydrolysis only yielded 3-O-galactopyranosyl [1]. The terminal hydrogen signals (δ 4.89 (1H, d, δJ = 8.0 Hz, H-1′), 5.35 (1H, d, δJ = 8.0 Hz, H-1′′)) suggested the presence of two β-β-glucopyranosyls. In its HMBC spectrum, long-range correlations from H-1′ to C-3, H-1’′ to C-3′ could be observed. As a result, their structures were finally elucidated to be 25(R)-5β-spirostan-3β-ol-12-one 3-O-β-β-apiofuranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-β-d-glucopyranoside (27), 25(R)-5β-spirostan-3β-ol-12-one 3-O-β-β-xylopyranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-β-d-glucopyranoside (27), 25(R)-5β-spirostan-3β-ol-12-one 3-O-β-β-xylopyranosyl (1→3)-[β-β-glucopyranosyl(1→6)]-β-β-d-glucopyranoside (38) and 25(R)-5β-spirostan-3β-ol-12-one 3-O-β-β-glucopyranosyl(1→3)-β-β-d-glucopyranoside (51), respectively. The appearance of a β-β-apiofuranosyl moiety provided new possibilities for speculation about the structure of other compounds.

These results not only confirmed the speculation based on chromatographic retention behaviors and the MS/MS cleavage patterns, but also made supplemented the assignment of the spiropstanol saponin glycosyl substituents of Y. schidigera.

In general, based on the phytochemistry researches reported before, this study accomplished a comprehensive chemical profiling of the spiropstanol saponins in Y. schidigera, especially, the targeted
isolation experiment made the analysis results more convincing. Moreover, the rules summarized in this paper also provided more accurate references to identify this kind of compounds.

3. Materials and Methods

3.1. Standard References for LC-MS/MS Research

Thirty-one spirostanol saponins, namely Yucca spirostanosides A1, A2, B1, B2, C1, C2, C3, D1, schidigera-saponin A1, schidigera-saponin A3, 5β-spirost-25(27)-en-3β-ol-12-one 3-O-β-D-glucopyranosyl(1→2)-O-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside, schidigera-saponin C2, schidigera-saponin C1 [1]; (25R)-Yucca spirostanoside E1, (25S)-Yucca spirostanoside E2, (25S)-Yucca spirostanoside E3, (25R)-Yucca spirostanoside F, (25R)-schidigera-saponin A, 25(R)-schidigera-saponin D5, 25(S)-schidigera-saponin D5, 25(R)-schidigera-saponin D1, 25(S)-schidigera-saponin D1 [6]; 25(S)-schidigera-saponin D9, 25(S)-schidigera-saponin E1, YS-VII, 25(S)-schidigera-saponin F2, 25(S)-schidigera-saponin F1 isolated from Y. schidigera by our lab were used as references. Their purities were >98%.

3.2. General Experimental Procedures, Materials and Methods

3.2.1. Materials and Reagents

Stems of Y. schidigera were collected from in the state of FL (USA) and identified by Dr. Li Tianxiang (The Hall of TCM Specimens, Tianjin University of TCM, Tianjin, China). A voucher specimen was deposited at the Academy of Traditional Chinese Medicine of Tianjin University of TCM (No. 20160301).

Acetonitrile (ACN), methanol (MeOH), formic acid (FA) of HPLC grade (Thermo, Waltham, MA, USA), ultra-pure water prepared with a Milli-Q purification system (Millipore, Billerica, MA, USA) and different HPLC columns [ACQUITY UPLC® BEH C18 (1.7 µm, 2.1 × 100 mm, Waters, Milford, MA, USA), ACQUITY UPLC® T3 (1.8 µm, 2.1 × 100 mm, Waters), ACQUITY UPLC® HSS C18 (1.8 µm, 2.1 × 100 mm, Waters), ACQUITY UPLC® C18 (1.8 µm, 2.1 × 100 mm, Waters)] were used for LC/MS analysis.

Analytical grade ethanol (EtOH), dichloromethane (CH2Cl2), methanol (MeOH) and macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), silica gel (48–75 µm, Haiyang Chemical Reagent Factory, Qingdao, China), ODS (40–63 µm, YMC Co., Ltd., Tokyo, Japan), MCI GEL CHP 20P (75–150 µm, Mitsubishi Chemical Holdings, Tokyo, JPN) were used for the preparation of Y. schidigera 70% EtOH extract (YS), silica gel fractionations of YS (YSESs), MCI gel fractionations of YS (YSEMs) test solutions and the separation of new spirostanol saponins. Preparative high-performance liquid chromatography (PHPLC) columns (Cosmosil 5C18-MS-II (20 mm i.d. × 250 mm, Nacalai Tesque, Inc., Kyoto, Japan), Wacopak Navi C30-5 (7.5 mm i.d. × 250 mm, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and Cosmosil PBr (20 mm i.d. × 250 mm, Nacalai Tesque, Inc.) were used for the separation of new spirostanol saponins.

Optical rotations were measured on a Rudolph Autopol® IV automatic polarimeter (l = 50 mm) (Rudolph Research Analytical, Hackettstown, NJ, USA). IR spectra were recorded on a Varian 640-IR FT-IR spectrophotometer (Varian Australia Pty Ltd., Mulgrave, Australia). Positive-ion mode ESI-Q-Exactive-Orbitrap MS were obtained on a Thermo Q Exactive Orbitrap MS spectrometer (Thermo). NMR spectra were determined on a Bruker 500 MHz NMR spectrometer (Bruker BioSpin AG Fällanden, Switzerland) at 500 MHz for 1H- and 125 MHz for 13C-NMR (internal standard: TMS).
3.2.2. Sample Preparation

Preparation of Standard Solutions

Standard test solutions of above-mentioned references were prepared in MeOH at a final concentration of approximately 100 ng/mL. All stock solutions were stored at 4 °C in darkness and brought to room temperature before use.

Preparation of YS Test Solutions

An aliquot of 1 kg dried powder of \textit{Y. schidigera} stems was extracted under reflux in 8, 6, 6 L 70% ethanol (\textit{v}/\textit{v}) for 3, 2, 2 h, respectively. The extract was combined and 50 mL was filtered with 0.22 µm microporous membrane to obtain YS test solutions. The rest of them was reserved as YS stock solution. YS test solutions were all stored at 4 °C in darkness and brought to room temperature before use.

Preparation of the YSEMs Test Solutions

The section “Preparation of YS Test Solutions” YS stock solution was evaporated under reduced pressure to obtain YS extract (160.0 g), which (150.0 g) was dissolved in 8 L of water and separated by D101 macroporous adsorption resin column (\textit{H}_2\text{O}→95\% \text{EtOH}) to obtain \textit{H}_2\text{O} eluates (80.2 g) and 95\% \text{EtOH} eluate (YSE, 60.7 g), respectively. YSE (30.0 g) were subjected to normal pressure MCI gel (200.0 g, 4.5 x 18 cm) column [\textit{H}_2\text{O}→95\% \text{EtOH}], fraction volume: 300 mL, nine fractions [YSEM1 (0.85 g), YSEM2 (1.32 g), YSEM3 (2.30 g), YSEM4 (5.45 g), YSEM5 (3.28 g), YSEM6 (1.00 g), YSEM7 (2.07 g), YSEM8 (5.12 g), YSEM9 (8.05 g)] were given. YSEM7–YSEM9 were evaporated to dryness under reduced pressure, and then dissolved with MeOH to get three test stock solutions (5 mg/mL) due to their enrichment of spirostanol saponin. YSEM7–YSEM9 stock solutions were stored at 4 °C in darkness and brought to room temperature before use.

Preparation of the YSESs Test Solutions

The section “Preparation of the YSEMs Test Solutions” YSE stock solution (30.0 g) was subjected to normal pressure silica gel (200.0 g, 4.5 x 30 cm, fraction volume: 500 mL) column [\textit{CH}_2\text{Cl}_2→\textit{CH}_2\text{Cl}_2→\text{MeOH} (100:1→100:3→100:7→5:1→3:1→2:1→0:100, \textit{v}/\textit{v})], and [YSES1 (0.22 g), YSES2 (1.06 g), YSES3 (1.61 g), YSES4 (0.88 g), YSES5 (0.69 g), YSES6 (6.91 g), YSES7 (5.57 g), YSES8 (5.85 g), YSES9 (6.66 g)] were obtained. YSES6–YSES9 stock solutions (5 mg/mL) were prepared by using the similar method as YSEM7–YSEM9 and stored at 4 °C in darkness and brought to room temperature before use.

3.2.3. Liquid Chromatography Setup

Separation of spirostanol saponins was performed on a Thermo UtiMate 3000 UHPLC instrument equipped with a quaternary pump, an autosampler. After the optimization of stationary phase (BEH C\textsubscript{18}, HSS C\textsubscript{18}, C\textsubscript{18} and T\textsubscript{3}), mobile phase (MeOH-H\textsubscript{2}O, ACN-H\textsubscript{2}O, MeOH-ACN-H\textsubscript{2}O), pH (0.1% and 0.01% FA) and column temperature (30 °C and 35 °C) (Figs S1–S4). Samples were separated on a Waters ACQUITY UPLC® T\textsubscript{3} (2.1 x 100 mm, 1.8 µm) using a mobile phase composed of H\textsubscript{2}O (A) and FA-MeOH-ACN (0.1:50:50, \textit{v}/\textit{v}/\textit{v}) (B) in the gradient program: 0–15 min, 12–24% B; 15–30 min, 24–32% B; 30–42 min, 32–40% B; 42–47 min, 40–60% B; 47–65 min, 60–95% B. An equilibration of 3 min was used between successive injections. The flow rate was 0.3 mL/min, and column temperature was 30 °C. An aliquot of 3 µL of each sample was injected for analysis.
3.2.4. ESI-Q-Exactive-Orbitrap MS High Resolution Tandem Mass Spectrometry and Automatic Components Extraction

Spirostanol saponins identification was carried out by using a Thermo ESI-Q-Exactive-Orbitrap MS (in the positive ESI mode (capillary voltage: 3.2 kV). Ultra-high purity nitrogen (N$_2$) and high purity nitrogen (N$_2$) were used as the collision gas and the sheath/auxiliary gas, respectively. The ESI source parameters were 350 °C for capillary temperature, 300 °C for ion source heater temperature, 40 arbitrary units for sheath gas (N$_2$), 10 arbitrary units for auxiliary gas (N$_2$), and the collision energy of the quadrupole ranged between 15 and 45 V were used. The mass range of the Orbitrap analyzer scanner was m/z 150 to 1500. Selected precursors analyzed more than two times were actively excluded from analysis for 60 s. Monitoring time was 0–65 min. Data recording and processing were performed using the Xcalibur 4.0 software (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The accuracy error threshold was fixed at 5 ppm. Automatic components extraction was accomplished by a Sieve v2.2 SP2 (Thermo Fisher Scientific) with the time range from 0 to 65 min and the BP minimum count at 10,000. Mass resolution is 70,000 and 17,500 for MS and MS$^2$, respectively.

3.2.5. Separation and Verification of the New Spirostanol Saponins Speculated by MPLC-MS/MS

The section “Preparation of the YSESs Test Solutions” fraction YSES6 (6.0 g) was separated by normal pressure ODS CC (40.0 g, 3 × 8 cm, fraction volume: 50 mL) [MeOH-H$_2$O (30:70→40:60→50:50→60:40→70:30→80:20→100:0, v/v)] and 14 fractions [Fr. 6-1 (150.0 mg), Fr. 6-2 (200.5 mg), Fr. 6-3 (325.2 mg), Fr. 6-4 (326.6 mg), Fr. 6-5 (126.6 mg), Fr. 6-6 (821.3 mg), Fr. 6-7 (435.5 mg), Fr. 6-8 (642.3 mg), Fr. 6-9 (164.8 mg), Fr. 6-10 (492.2 mg), Fr. 6-11 (298.3 mg), Fr. 6-12 (300.5 mg), Fr. 6-13 (542.5 mg), Fr. 6-14 (1100.5 mg)]. Fraction 6-11 (298.3 mg) was subjected to PHPLC [MeOH-H$_2$O (75:25, v/v) + 1% HAc, Cosmosil 5C$_{18}$-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] to obtain (25R)-5β-spirostan-3β,12β-diol 3-O-β-D-xylopyranosyl(1→3)-β-D-glucopyranoside (34/36, 11.7 mg). Fraction 6-11-5 (28.7 mg) was subjected to silica gel CC [CH$_2$Cl$_2$-MeOH (100:7, v/v)] to obtain 25(S/R)-5β-spirostan-3β,12β-diol 3-O-β-D-glucopyranoside (40/41, 11.9 mg) was given. Fraction 6-12 (400.5 mg) was purified by PHPLC [MeOH-H$_2$O (75:25, v/v) + 1% HAc, Cosmosil 5C$_{18}$-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] to obtain (25R)-5β-spirostan-3β-ol-12-one 3-O-β-D-glucopyranosyl(1→3)-β-D-glucopyranoside (51, 3.3 mg).

The section “Preparation of the YSESs Test Solutions” fraction YSES7 (5.0 g) was subjected to PHPLC [MeOH-H$_2$O (80:20, v/v) + 1% HAc, Cosmosil 5C$_{18}$-MS-II column (20 mm i.d. × 250 mm), 9 mL/min], and 12 fractions [Fr. 7-1 (1010.1 mg), Fr. 7-2 (850.4 mg), Fr. 7-3 (253.8 mg), Fr. 7-4 (223.2 mg), Fr. 7-5 (356.3 mg), Fr. 7-6 (36.5 mg), Fr. 7-7 (223.2 mg), Fr. 7-8 (42.3 mg), Fr. 7-9 (76.2 mg), Fr. 7-10 (492.3 mg), Fr. 7-11 (104.0 mg), Fr. 7-12 (800.1 mg)] were obtained. Fraction 7-5 (356.3 mg) was separated by PHPLC [MeOH-H$_2$O (70.30, v/v) + 1% HAc, Cosmosil 5C$_{18}$-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] to afford (25S)-5β-spirostan-3β-ol-12-one 3-O-β-D-apiofuranosyl(1→3)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (27, 9.6 mg).

The section “Preparation of the YSESs Test Solutions” fraction YSES8 (5.0 g) was separated by PHPLC [MeOH-H$_2$O (80:20, v/v) + 1% HAc, Cosmosil 5C$_{18}$-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] to yield 17 fractions [Fr. 8-1 (2380.7 mg), Fr. 8-2 (136.2 mg), Fr. 8-3 (370.2 mg), Fr. 8-4 (309.7 mg), Fr. 8-5 (92.4 mg), Fr. 8-6 (212.1 mg), Fr. 8-7 (89.7 mg), Fr. 8-8 (48.1 mg), Fr. 8-9 (60.1 mg), Fr. 8-10 (133.1 mg), Fr. 8-11 (43.1 mg), Fr. 8-12 (10.7 mg), Fr. 8-13 (22.1 mg), Fr. 8-14 (16.3 mg), Fr. 8-15 (40.2 mg), Fr. 8-16 (26.1 mg), Fr. 8-17 (700.0 mg)]. Fraction 8-5 (92.4 mg) was subjected to PHPLC [ACN-H$_2$O (38:62, v/v) + 1% HAc, Cosmosil 5C$_{18}$-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] and PHPLC [MeOH-H$_2$O (60:40, v/v) + 1% HAc, Cosmosil PBr column (20 mm i.d. × 250 mm), 9 mL/min] successively to obtain 25(S/R)-5β-spirostan-3β,12β-diol 3-O-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (14/15, 2.9 mg). Fraction 8-13 (22.1 mg) was isolated by PHPLC [MeOH-H$_2$O (78:22, v/v) + 1% HAc, Wakopak Navi C$_{30}$-5 column (7.5 mm i.d. × 250 mm), 3 mL/min], and (25R)-5β-spirostan-3β-ol-12-one 3-O-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (38, 13.5 mg) was obtained.
The section “Preparation of the YSEsS Test Solutions” fraction YSES9 (6.0 g) was isolated by PHPLC [MeOH-H2O (80:20, v/v) + 1% HAc, Cosmosil 5C18-M-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] to obtain 16 fractions [Fr. 9-1 (2070.3 mg), Fr. 9-2 (163.2 mg), Fr. 9-3 (115.1 mg), Fr. 9-4 (175.9 mg), Fr. 9-5 (160.1 mg), Fr. 9-6 (175.4 mg), Fr. 9-7 (110.1 mg), Fr. 9-8 (297.2 mg), Fr. 9-9 (112.6 mg), Fr. 9-10 (150.0 mg), Fr. 9-11 (81.7 mg), Fr. 9-12 (147.3 mg), Fr. 9-13 (107.0 mg), Fr. 9-14 (33.6 mg), Fr. 9-15 (75.9 mg), Fr. 9-16 (1650.3 mg)]. Fraction 9-7 (110.1 mg) was separated successively by PHPLC [MeOH-H2O (85:15, v/v) + 1% HAc, Cosmosil 5C18-M-MS-II column (20 mm i.d. × 250 mm), 9 mL/min] and PHPLC [ACN-H2O (32:68, v/v) + 1% HAc, Wakopak Navi C50-5 column (7.5 mm i.d. × 250 mm), 3 mL/min] to obtain 25(R)-5β-spirostan-2β,3β-diol-12-one-3-O-xylopyranosyl(1→3)-[glucopyranosyl (1→2)]galactopyranoside (10, 9.2 mg).

(25R)-5β-spirostan-2β,3β-diol-12-one-3-O-β-d-glucopyranosyl(1→3)-[β-n-glucopyranosyl(1→2)]-β-n-galactopyranoside (10): White powder; [α]D25: −7.7° (conc. 0.62, MeOH); IR νmax (KBr) cm⁻¹: 3353, 2928, 2872, 1704, 1455, 1377, 1159, 1074, 1043, 983; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S1 ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 903.4550 [M + H]+ (calcd for C44H71O19, 903.4584).

25(S/R)-5β-spirostan-3β,12β-diol-3-O-β-d-glucopyranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-n-galactopyranoside (14/15): White powder; [α]D25: −8.0° (conc. 0.34, MeOH); IR νmax (KBr) cm⁻¹: 3398, 2930, 2876, 1644, 1453, 1373, 1160, 1077, 1043, 990; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S2; ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 889.4799 [M + H]+ (calcd for C44H73O18, 889.4791).

(25R)-5β-spirostan-3β-ol-12-one-3-O-β-d-glucopyranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-n-galactopyranoside (27): White powder; [α]D25: −10.3° (conc. 0.79, MeOH); IR νmax (KBr) cm⁻¹: 3412, 2929, 2874, 1705, 1453, 1161, 1079, 1028, 982; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S3; ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 887.4658 [M + H]+ (calcd for C44H71O18, 887.4635).

25(S/R)-5β-spirostan-3β,12β-diol-3-O-β-d-glucopyranosyl(1→3)-[β-β-glucopyranosyl(1→2)]-β-n-galactopyranoside (34/36): White powder; [α]D25: −36.0° (conc. 0.050, MeOH); IR νmax (KBr) cm⁻¹: 3396, 2928, 2869, 1646, 1546, 1453, 1372, 1161, 1045, 986; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S4; ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 727.4282 [M + H]+ (calcd for C38H43O13, 727.4263).

(25R)-5β-spirostan-3β-ol-12-one-3-O-β-d-glucopyranosyl(1→3)-[β-β-glucopyranosyl(1→6)]-β-n-galactopyranoside (38): White powder; [α]D25: −16.2° (conc. 0.78, MeOH); IR νmax (KBr) cm⁻¹: 3423, 2930, 2874, 1705, 1456, 1377, 1164, 1073, 1039, 995; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S5; ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 887.4632 [M + H]+ (calcd for C44H73O18, 887.4635).

25(S/R)-5β-spirostan-3β,12β-diol-3-O-β-d-glucopyranoside (40/41): White powder; [α]D25: −44.7° (conc. 0.085, MeOH); IR νmax (KBr) cm⁻¹: 3414, 2930, 2871, 1646, 1563, 1452, 1376, 1166, 1061, 1022, 990; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S6; ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 595.3847 [M + H]+ (calcd for C35H35O6, 595.3841).

(25R)-5β-spirostan-3β-ol-12-one-3-O-β-n-glucopyranosyl(1→3)-β-n-galactopyranoside (51): White powder; [α]D25: −0.3° (conc. 0.68, MeOH); IR νmax (KBr) cm⁻¹: 3388, 2928, 2871, 1706, 1453, 1375, 1159, 1077, 1036, 986; 1H-NMR (C5D5N, 500 MHz) and 13C-NMR (C5D5N, 125 MHz) data δ: see Table S7; ESI-Q-Exactive-Orbitrap MS positive-ion mode m/z 755.4223 [M + H]+ (calcd for C39H43O14, 755.4212).

4. Conclusions

In conclusion, in order to provide references for the quality assessment standard establishment of Y. schidigera, a MPLC-MS/MS analysis technique was used to accomplish the qualitative analysis of the spirostanol saponins from its extract, and a comprehensive characterization method for spirostanol saponins was set up for the first time. This will lay a foundation for the quality evaluation of Y. schidigera.
On the other hand, the chromatographic retention behaviors and the MS/MS cleavage patterns of spirostanol saponins were summarized.

In summary, according to the retention time ($t_R$) and the exact mass-to-charge ratio ($m/z$), thirty-one compounds were unambiguously identified by comparing them to references. Meanwhile, the MS/MS fragmentation pattern and chromatographic elution order rules have been generalized by using the standard compounds as references, seventy-nine compounds were tentatively identified and forty of them were potential new ones. Among them, ten were targeted for separation to prove the correctness of our speculations. During the verification process, the appearance of a β-$\nu$-apiofuranosyl moiety was found for the first time, which provided new possibilities for speculation about the structure of other compounds. As a result, an accurate and comprehensive chemical composition profiling of the aerial part of the Y. schidigera was realized, which lays a foundation for the quality evaluation of the plant.

**Supplementary Materials:** Supplementary data (Materials and Methods section, BPCs for conditional optimization experiment, 1D, 2D NMR, and HRMS/MS spectra of compounds (10, 14, 15, 27, 34, 36, 38, 40, 41 and 51). The summary of characteristic types of substituted glycosyl groups, characteristic fragment ions of seven aglycone moieties and the chromatographic elution order of spirostanol saponins from YSSs aglycons.) associated with this article can be found in the online version.

**Author Contributions:** Y.Z. and T.W. designed the research and wrote the manuscript; J.R., L.Q., and W.Z. performed the experimental work; C.G., P.H. and D.Z. corrected the data and reviewed the literatures; L.H., H.Y. and Z.Z. perfected the language. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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**Sample Availability:** Samples of the compounds “Yucca spirostanosides A1, A2, B1, B2, B3, C1, C2, C3, D1, schidigera-saponin A1, schidigera-saponin A3, 5β-spirost-25(27)-en-3β-ol-12-one-3-O-β-D-glucopyranosyl(1→2)-O-[(β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside, schidigera-saponin C2, schidigera-saponin C1, (25R)-Yucca spirostanoside E1, (25S)-Yucca spirostanoside E1, (25R)-Yucca spirostanoside E2, (25S)-Yucca spirostanoside E2, (25R)-Yucca spirostanoside E3, (25S)-Yucca spirostanoside E3, (25R)-5β-spirostan-3β-ol-3-O-β-D-glucopyranoside, asparagoside A, 25(S)-schidigera-saponin D5, 25(S)-schidigera-saponin D5, 25(R)-schidigera-saponin D1, 25(S)-schidigera-saponin D1, 25(S)-schidigera-saponin D3, 25(S)-schidigera-saponin E1, YS-VII, 25(S)-schidigera-saponin F2, 25(S)-schidigera-saponin F1” are available from the authors.

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