A comprehensive and comparative analysis of liposoluble constituents in sloughs of five different species of snakes by GC-MS

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Abstract. As a sort of traditional Chinese medicine (TCM), snake sloughs have been proved to be effective in curing miscellaneous diseases such as pruritus, muscular spasm and laryngalgia. However, there are few researches on their chemical components, especially the discriminant analysis based on different species. In this study, the liposoluble constituents in sloughs of five species of snakes (Deinagkistrodon, Elaphe carinata, Naja atra, Ptyas mucosus and Zaocys dhumnades) were revealed by comparative evaluation on GC-MS analysis that fatty acids (49.89%-54.65%) and steroids (13.55%-24.98%) were their major components. Moreover, the content of polyunsaturated fatty acids was found by cluster analysis to be the key index to distinguish the quality of a snake slough. It could be concluded that the slough of Naja atra be more appropriate to be a raw material of traditional Chinese medicines. It was also concluded that the GC-MS based method as well as the experimental results could be of referential value for further studies on the efficiency and activities of snake sloughs.

1. Introduction

Snakes are reptilian creatures that distribute wildly in tropical and subtropical regions, including south and southeast of China. Most of the snakes, according to the variety of species, slough 2-10 times per year. The snake sloughs, also known as the shed skins of snakes, are considered as a valuable traditional Chinese medicine (TCM) and have already been proved by clinical investigations to be efficacious against pruritus, muscular spasm, laryngalgia, etc [1], therefore the researches on the chemical components in snake sloughs are obviously necessary. However, only a few reports can be found in this field [2-4]. For instance, thirty eight fatty acids and seventeen amino acids were detected in the shed skin of Bungarus multicinctus [4], but no further studies had been carried out. Since the medicinal efficiency of sloughs would be discrepant based on the species of snakes, a comparative analysis of the chemical constituents of different snakes is of importance, and thus we focused on the determination of liposoluble constituents, including the fatty acids, esters, alcohols, steroids et al, so as to facilitate further researches on the structural composition as well as the medicinal mechanism of snake sloughs.

The chemical constituents of snake sloughs can be determined by chromatography, such as high/ultra performance liquid chromatography (HPLC/UPLC) [5-7], gas chromatography-mass spectroscopy (GC-MS) [8-11], thin layer chromatography (TLC) [12, 13] and so on. In our study, sloughs of five of the most common snakes in China, including Deinagkistrodon, Elaphe carinata, Naja atra, Ptyas mucosus and Zaocys dhumnades, were collected early in 2016 and their liposoluble constituents [14, 15]
were identified by GC-MS due to its advantages in simultaneous qualitative and quantitative analysis. Cluster analysis was taken out afterwards to evaluate the differences between the five species of sloughs.

2. Experimental

2.1. Materials
Sloughs of five species of snakes, including *Deinagkistrodon* (labelled SS1), *Elaphe carinata* (SS2), *Naja atra* (SS3), *Ptyas mucosus* (SS4) and *Zaocys dhumnades* (SS5) were collected directly from snake keepers in Hunan, Guangdong and Anhui province (Table 1).

| Sample label | Species           | Toxic/nontoxic | Habitat                          | Province   |
|--------------|-------------------|----------------|----------------------------------|------------|
| SS1          | *Deinagkistrodon* | Toxic          | Deciduous forests and ditches     | Guangdong  |
| SS2          | *Elaphe carinata* | Nontoxic       | Plains and hills                 | Anhui      |
| SS3          | *Naja atra*       | Nontoxic       | Jungles and bamboo groves        | Hunan      |
| SS4          | *Ptyas mucosus*   | Toxic          | Plains and hills                 | Anhui      |
| SS5          | *Zaocys dhumnades*| Nontoxic       | Hills                            | Anhui      |

2.2. Reagents
All chemical reagents used in the experiment are listed in Table 2.

| Reagent                    | Type               | Manufacturer                                  |
|----------------------------|--------------------|-----------------------------------------------|
| n–Hexane                   | Analytical reagent | Sigma–Aldrich Co. LLC.                        |
| Acetic ester               | Analytical reagent | Aladdin Industrial Corporation                |
| Aether                     | Analytical reagent | Tianjin Kangkede Technology Co. Ltd.         |
| Potassium hydrate          | Analytical reagent | Tianjin Kangkede Technology Co. Ltd.         |
| Ethanol                    | Analytical reagent | Aladdin Industrial Corporation                |
| Hydrochloric acid          | Analytical reagent | Tianjin Kangkede Technology Co. Ltd.         |
| Anhydrous sodium sulfate   | Analytical reagent | Aladdin Industrial Corporation                |
| 99% Bis (trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) | Derivatizing reagent | Tokyo Chemical Industry |

2.3. Pretreatments of samples
After dehydration and smashing, 20 g of each sample was immersed in 400 mL of elute solution (n-Hexane: ethyl ester = 1:1), respectively. With condensate water on, each of the mixtures was heated to 85 °C and kept for 7 hours in water bath before cooled down to room temperature (22 °C) and filtered. All solvents were evaporated by heating the filtrate to 70 °C in a ventilation environment to afford an amber oily substance. Then this substance was added 65 mL saturated potassium hydrate-ethanol, and the system was set in a water bath at 60 °C for 1 hour. The mixture was diluted with 100 mL of distilled water and extracted by 50 mL of ether in a separating funnel. The aqueous phase was adjusted to pH 2.00 ± 0.02 by hydrochloric acid and extracted by another 50 mL of ether. The organic layers were combined, dried over anhydrous sodium sulfate and heated to remove the solvent to afford a light brown insoluble substance.

Insoluble substance (from oil phase) of about 2.0 mg mixed with 100 μL of 99% BSTFA + 1% TMCS was water bathed at 55 °C for 1.5 hours. All samples were ready for GC-MS analysis after derivatization and centrifugation (12000 r/min, 5 min)

2.4. Analysis of GC-MS
The GC-MS analysis was performed on a GC-2010 plus gas chromatograph (Shimadzu, Japan) equipped with a TQ8030 mass spectrometer (Shimadzu, Japan) and a MP–5ms gas chromatographic column (0.25 mm x 30 m x 0.25 μm, Agilent, USA).
The temperature of the injector was set at 280 °C and the pressure was 73.0 kPa, flow rate of the carrier gas (nitrogen) was 1.00 mL/min and the split ratio was 20:1. The temperature of the column was set to keep at 100 °C for 3 min firstly, then raised to 300 °C at 8 °C/min and kept for 6 min. The injection volume was 1.0 μL.

As for the mass spectrometer, the temperature of the ion source was 230 °C. Scan mode was Q3 and the range of mass-to-charge ratio (m/z) was from 35.00 to 800.00.

2.5. Data Analysis
Identification of the compounds were performed by comparing the retention indices and mass spectra to standard library NIST 11, and the relative concentration of each constituent was determined by calculating the peak areas in total ion current (TIC). Cluster analysis was carried out using The Unscrambler (version 10.3, CAMO Software, Norway), the Ward’s method was selected to be the algorithms while squared Euclidean distance was applied to evaluate the similarity [8, 16, 17].

3. Test Results and Discussions
The TIC of liposoluble constituents of five samples are shown in Fig. 1.

![Fig. 1](image)

Fig. 1  Total ion current (TIC) of liposoluble constituents in snake sloughs, (a) Deinagkistrodon, (b) Elaphe carinata, (c) Naja atra, (d) Ptyas mucosus, (e) Zaocys dhumnades.
A number of 141 compounds were confirmed in the five samples. SS2 and SS3 were the richest in components (79 in each) while SS5 was the fewest (66).

According to the statistics listed in Table 3, fatty acids (FAs) and steroids were the main compounds in snake sloughs. The contents of total FAs in five samples were generally similar, varying slightly from 49.89% (SS4) to 54.65% (SS5), but the percentage of saturated fatty acids (SFAs) and unsaturated fatty acids (USFAs) were quite different. It is remarkable that SS5 was particularly rich in SFAs while the concentration in the others was comparatively in a lower level. Octadecanoic acid (stearic acid), hexadecanoic acid (palmitic acid), eicosanoic acid (arachic acid) and docosanoic acid (behenic acid) accounted the vast majority of SFAs. Odd-carbon SFAs, such as pentadecanoic acid, heptadecanoic acid and nonadecanoic acid, were also detected though they were less in the even-carbon ones. The most common monounsaturated fatty acids (MUFAs) were cis-9-octadecenoic acid (oleic acid) and its trans isomer, both of which had been detected widely in animal tissues. This couple of octadecenoic acids made up the main composition of MUFAs in all samples. As for the polyunsaturated fatty acids (PUFAs), 9,12-octadecadienoic acid (linoleic acid), 8,11,14-eicosatrienoic acid and 5,8,11,14-tetraenoic (arachidonic acid, ARA) acid are the common ones, SS2, SS3 and SS4 were obviously higher in total (all above 10%), indicating that they should be more efficient in antioxidation and free radical eliminating than the other two. Note that the content of ARA in SS3 was extremely high (2.33%) and 4,7,10,13,16,19-docosahexaenoic (DHA) acid was only detected in this sample, which could be considered as a characteristic of Naja atra sloughs.

Table 3  Concentration of the compounds calculated based on relative peak areas (%)

| Compound No. | acids                        | SS1 | SS2 | SS3 | SS4 | SS5 |
|-------------|------------------------------|-----|-----|-----|-----|-----|
| 1           | Butanoic acid                | – a | 0.01| 0.11| 0.04| 0.31|
| 2           | 3–methyl Butanoic acid b     | –   | –   | –   | –   | 0.05|
| 3           | 2–oxo–Butanoic acid          | –   | –   | 0.06| 0.01| 0.08|
| 4           | Pentanoic acid               | 0.02| 0.02| 0.20| 0.36| –   |
| 5           | 4–methyl Pentanoic acid      | 0.06| –   | –   | –   | –   |
| 6           | Hexanoic acid                | 0.17| 0.30| 0.05| 0.32| –   |
| 7           | 2–oxo–Hexanoic acid          | –   | 0.02| –   | –   | –   |
| 8           | Heptanoic acid               | 0.02| 0.02| –   | –   | –   |
| 9           | Octanoic acid                | 0.13| –   | 0.02| –   | –   |
| 10          | Nonanoic acid                | 0.02| 0.04| 0.05| 0.01| 0.02|
| 11          | Decanoic acid                | 2.52| –   | 0.48| –   | 0.30|
| 12          | 3–hydroxy Decanoic acid      | 0.32| –   | –   | –   | –   |
| 13          | Hendecanoic acid             | 0.04| –   | –   | –   | –   |
| 14          | Dodecanoic acid              | 0.08| 0.02| 0.02| 0.01| –   |
| 15          | Tridecanoic acid             | 0.04| 0.02| –   | –   | 0.02|
| 16          | Tetradecanoic acid           | 0.76| 0.12| 0.19| 0.04| 0.08|
| 17          | Pentadecanoic acid           | 1.66| 0.13| 0.48| 0.23| 0.14|
| 18          | hexadecanoic acid            | 5.63| 8.09| 4.93| 6.36| 5.04|
| 19          | Heptadecanoic acid           | 1.00| 0.12| 0.82| 1.19| 0.32|
| 20          | Octadecanoic acid            | 7.90| 4.81| 9.28| 4.61| 12.79|
| 21          | Nonadecanoic acid            | 0.68| 0.58| 1.64| 0.68| 1.10|
| 22          | Eicosanoic acid              | 3.89| 5.00| 4.72| 5.75| 8.82|
| 23          | Docosanoic acid              | 3.73| 1.72| 4.08| 3.52| 8.74|
| 24          | Tetracosanoic acid           | –   | 0.11| –   | –   | –   |
| 25          | Benzoic acid                 | –   | 0.01| 0.01| 0.01| 0.05|
| 26          | Benzene acetic acid          | –   | 0.02| 0.47| 0.07| –   |
| 27 | Benzene propanoic acid | 0.20 | 0.21 |  
| 28 | 2-Benzenepropanoic acid | 0.02 |  
| 29 | Ethane dioic acid |  
| 30 | Propanedioic acid | 0.03 |  
| 31 | methyl–Propanedioic acid |  
| 32 | Butanedioic acid |  
| 33 | methyl–Butanedioic acid |  
| 34 | Pentanedioic acid |  
| 35 | Hexanedioic acid |  
| 36 | Heptanedioic acid |  
| 37 | Octanedioic acid |  
| 38 | Nonanedioic acid |  
| 39 | Decanedioic acid |  
| 40 | Dodecanedioic acid |  
| 41 | Pentenoic acid |  
| 42 | 3–Pentenoic acid |  
| 43 | 4–Pentenoic acid |  
| 44 | 10–Undecenoic acid |  
| 45 | 6–Octadecenoic acid |  
| 46 | cis–9–Octadecenoic acid | 15.91 | 4.26 | 11.59 | 13.57 |  
| 47 | trans–9–Octadecenoic acid | 2.04 | 1.02 | 1.22 | 0.58 |  
| 48 | 6,9–Octadecadienoic acid |  
| 49 | 9,12–Octadecadienoic acid |  
| 50 | 9,12,15–Octadecatrienoic acid |  
| 51 | 8,11,14–Eicosatrienoic acid |  
| 52 | 5,8,11,14–Tetraenoic acid |  
| 53 | 4,7,10,13,16,19–Docosahexaenoic acid |  
| 54 | Butanol |  
| 55 | 2–Butanol | 0.06 |  
| 56 | 3–methyl Butanol |  
| 57 | 3,7–dimethyl Octanol |  
| 58 | Decanol |  
| 59 | 2–Decanol |  
| 60 | 2–hexyl–Decanol |  
| 61 | Dodecanol | 0.02 |  
| 62 | Tetradecanol |  
| 63 | Pentadecanol |  
| 64 | Hexadecanol |  
| 65 | Heptadecanol |  
| 66 | Octadecanol |  
| 67 | Hexacosanol |  
| 68 | Octacosanol |  
| 69 | 9,12–Octadecadienol |  
| 70 | 3,7,11,15–tetramethylhexadec–2–enol |  
| 71 | Octade–9–enol |  
| 72 | Ethanediol | 0.04 | 0.05 |  
| 73 | Octadec–9–enol |  
| 74 | Ethanediol | 0.04 | 0.05 |  
| 75 | Octadec–9–enol |  
| 76 | Ethanediol | 0.04 | 0.05 |  

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|    | Butane–2,3–dil  |     |     |     |     |
|----|-----------------|-----|-----|-----|-----|
| 73 | Glycerol        | 0.01|     |     |     |
| 74 | 1–monooxyglycer | 0.12| 0.10| 0.19| 0.23|
| 75 | 1–O–heptadecyl Glycerol | 5.80| 9.14| 4.33| 6.01|
| 76 | Butane–1,2,4–triol |     |     |     | 0.06|
| 77 | **alkanes**     |     |     |     |     |
| 78 | 2,4–dimethyl–Hexane |     |     |     | 0.18|
| 79 | 2,4,4–trimethyl–Hexane |     |     | 0.14|     |
| 80 | 4–ethyl–Heptane  |     |     |     |     |
| 81 | 3,3–dimethyl–Heptane |     |     | 0.10|     |
| 82 | 3–ethyl–3–methyl–Heptane |     |     |     | 0.10|
| 83 | 3,3,5–trimethyl–Heptane |     |     |     |     |
| 84 | 2,2,3,3,5,6,6–heptamethyl–Heptane |     |     |     |
| 85 | 3,3–dimethyl–Octane | 0.08|     | 0.05| 0.41| 0.15|
| 86 | 5–butyl–Nonane   | 1.15|     |     | 0.07| 0.06|
| 87 | n–Undecane      | 0.06| 0.06| 0.05| 0.13| 0.09|
| 88 | 5–methyl–Undecane |     |     |     |     |
| 89 | 2,6–dimethyl–Undecane | 0.05|     |     |     |
| 90 | n–Dodecane      | 0.34| 0.18|     | 1.20| 1.58|
| 91 | 2–methyl–Dodecane |     | 0.08|     |     |
| 92 | 4,6–dimethyl–Dodecane | 0.10|     |     |     |
| 93 | 2,6,10–trimethyl–Dodecane | 0.65| 0.34| 0.46| 0.35| 0.47|
| 94 | 2,6,11–trimethyl–Dodecane |     | 0.95|     |     |
| 95 | n–Tridecane     | 0.12| 0.05| 0.86| 0.08| 0.07|
| 96 | n–Tetradecane   |     |     | 0.24|     |
| 97 | n–Pentadecane   | 0.14|     |     | 0.04|     |
| 98 | 2–methyl–Pentadecane |     |     | 0.01|     |
| 99 | n–Hexadecane    | 2.18| 2.17| 2.76| 1.95| 2.35|
| 100| 2,6,10,14–tetramethyl–Hexadecane | 0.20| 0.16| 0.07| 0.09| 0.15|
| 101| n–Heptadecane   | 0.85| 0.17|     | 0.13|     |
| 102| 8–methyl–Heptadecane |     | 0.08|     |     | 0.03|
| 103| n–Nonadecane    | 0.22| 0.16| 0.24| 0.62| 0.28|
| 104| n–Heneicosane   | 0.53| 0.48| 0.28| 0.36| 0.57|
| 105| n–Tetracosane   | 0.07| 0.11|     | 0.07|     |
| 106| n–Octacosane    | 0.44| 0.49| 0.43| 0.40| 0.73|
| 107| n–Nonacosane    | 0.07| 0.12| 0.04|     |
| 108| n–Pentatriacontane |     | 0.05|     |     | 0.06|
| 109| n–Tetracontane  | 0.11| 0.06|     |     |
| 110| n–Tetratetracontane | 0.05| 0.01| 0.12| 0.04| 0.33|
| 111| n–Tetrapentacontane |     | 0.10|     |     |

|    | **esters**      |     |     |     |     |
| 112| 2–methyl–Butanoic acid hexyl ester |     |     | 0.06| 0.06|
| 113| Docosanoic acid methyl ester        | 0.22| 0.22| 0.17| 0.32|
| 114| Tetracosanoic acid methyl ester     | 1.88|     | 0.01|     |
| 115| Dodecyl acrylate                    |     | 0.06| 0.08| 0.03|
| 116| 7,10–Hexadecadienoic acid methyl ester | 0.90| 4.43|     |     |
| 117| 11,14–Eicosadienoic acid methyl ester |     | 2.00|     |     |
| 118 | 5,8,11,14–Eicosatetraenoic acid ethyl ester | – | – | – | 0.14 | – |
| 119 | Phthalic acid bis (7–methyloctyl) ester | – | – | 0.02 | 0.01 | 0.02 |
| 120 | Isocitric lactone | 0.02 | – | – | – | – |
| 121 | Isopropyl linoleate | – | – | 2.39 | – | – |
| 122 | Oleyl oleate | – | – | 4.12 | – | – |

**Steroids**

| 123 | Cholesterol | 14.72 | 13.66 | 10.12 | 13.73 | 18.36 |
| 124 | Cholestane | 2.03 | 3.18 | 2.33 | 1.72 | 3.27 |
| 125 | Cholest–5–ene | 1.66 | 3.12 | 0.79 | 2.85 | 3.17 |
| 126 | Cholest–5–en–3–ol | 0.12 | – | – | – | – |
| 127 | Cholesterol epoxide | 0.23 | – | – | – | – |
| 128 | Sitosterol | 1.18 | 0.28 | 0.31 | 1.51 | 0.18 |
| 129 | Stigmasterol | 0.16 | – | – | – | – |

**Amines and amides**

| 130 | Ethylamine | 0.32 | – | 0.77 | 0.23 | 1.13 |
| 131 | Diethylamine | – | – | 0.01 | – | 0.05 |
| 132 | 2–methyl Cyclohexylamine | – | 0.23 | – | – | – |
| 133 | Phenylethanamine | 0.01 | – | – | – | – |
| 134 | Hexadecanamide | – | – | – | – | 0.16 |
| 135 | N–ethyl–Acetamide | – | 0.04 | 0.04 | 0.01 | 0.07 |
| 136 | N–acetyl–N–methyl–Acetamide | 0.02 | – | – | – | – |

**Others**

| 137 | hexadecyl–Oxirane | – | 0.18 | 4.34 | 3.09 | 2.60 |
| 138 | 1,4–Dioxane | – | 0.04 | – | – | – |
| 139 | Borate | 0.03 | 0.13 | 0.06 | 0.08 | 0.05 |
| 140 | 7–Hexadecenal | 0.48 | – | 0.95 | – | – |
| 141 | 9–Tetradecenal | – | 0.59 | – | – | – |

| Total | 53.42 | 50.70 | 53.86 | 49.89 | 54.65 |
|-------|-------|-------|-------|-------|-------|
| Acids |       |       |       |       |       |
| Saturated fatty acids | 28.29 | 21.11 | 27.07 | 23.12 | 37.68 |
| Substituted fatty acids | 0.38 | 0.02 | 0.06 | 0.01 | 0.13 |
| Monounsaturated fatty acids | 18.92 | 15.11 | 10.82 | 12.83 | 14.24 |
| Polyunsaturated fatty acids | 5.57 | 13.28 | 14.83 | 13.26 | 2.02 |
| Aromatic acid | – | 0.02 | 0.70 | 0.29 | 0.05 |
| Dioic acids | 0.26 | 1.16 | 0.38 | 0.38 | 0.53 |
| Alcohols | 6.84 | 16.14 | 7.31 | 11.26 | 5.27 |
| Alkanes | 7.31 | 5.86 | 5.89 | 6.33 | 7.52 |
| Esters | 3.02 | 4.77 | 8.70 | 0.62 | 0.85 |
| Steroids | 20.10 | 20.24 | 13.55 | 19.81 | 24.98 |
| Amines and Aides | 0.35 | 0.27 | 0.82 | 0.24 | 1.41 |
| Others | 0.51 | 0.94 | 5.35 | 3.17 | 2.65 |
| Compounds Identified | 91.55 | 98.92 | 95.48 | 91.32 | 97.33 |

\(a\) (–): not detected.
\(b\) Compounds in each group are sorted according to the number of carbon atoms in main chains.

Steroids, mostly cholesterol and its derivatives, are the second abundant component in snake sloughs (13.55% to 24.98%). Cholest-5-en-3-ol(0.12%), cholesterol epoxide(0.23%) and stigmasterol(0.16%)
were the characteristic compounds of SS1. Additionally, it could be observed that the content of cholesterol was inversely correlative with that of the PUFAs, the results was consistent with previous theories that PUFAs had the effect in reducing cholesterols [18].

Alcohols were less popular in all samples. Though 24 alcohols were found, only a few of them were mutual. Besides a small amount of saturated monohydric alcohols (0.24%-2.02% in total), glycerol and its derivatives (mainly 1-O-heptadecyl glycerol) were evidently higher in content. Unsaturated alcohols, including 3, 7, 11, 15-tetramethylhexadec-2-enol and octadec-9-enol exist in SS2, SS3 and SS4 only, and no unsaturated alcohols were determined in SS1 and SS5.

Most of the alkanes detected in samples were straight-chained hydrocarbons and n-hexadecane (1.95%-2.35%), n-octacosane (0.40%-0.73%), n-heneicosane (0.28%-0.57%) and n-tridecane (0.05%-0.86%) were the major ones. Long-chained alkanes (n > 30) were also proved to be existed in snake sloughs and SS2 contained the most types, including n-pentatriacontane (0.05%), n-tetracontane (0.11%), n-tetracontacontane (0.01%) and n-tetrapentacontane (0.10%). Except 2,6,10-trimethyl-dodecane (0.34%-0.65%) and 2,6,10,14-tetramethyl-hexadecane (0.07%-0.20%), branched alkanes distributed randomly and thus difficult to summarize a regulation.

Some other compounds could be regarded as the characteristic components due to their specificity, such as 9-tetradecenal in SS2 (0.59%) and hexadecanamide in SS5 (0.16%). Meanwhile, there were also some chemical structures could not be considered as constituents in snake sloughs though they were detected in samples. For example, 1,7,7-trimethyl-bicyclo [2.2.1] heptan-2-one was detected in SS1 (0.01%) during our experiment but was manually excluded from Table 3 because it was more likely from the an environment of camphor where a Deinagkistrodon sloughed.

As for the cluster analysis, all 5 samples were divided into two groups according to the chemical compositions (Fig. 2). SS5 and SS1 were in one cluster while SS3, SS4 and SS2 were in another. Moreover, SS4 and SS2 were disposed together and SS3 was alone in a sub-cluster. It could be concluded that the result of the cluster analysis was related closely to the content of PUFAs as well as cholesterol. SS3 has the largest amount of PUFAs and the lowest cholesterol level, which meant the slough of a Naja atra would be the most appropriate raw material for a TCM prescription. By contrast, Deinagkistrodon and Zaocys dhumnade sloughs would probably not be suitable.

![Cluster analysis by The Unscrambler (v10.3), squared Euclidean distance was chosen to be the evaluating index of similarity.](image-url)

Fig. 2  Cluster analysis by The Unscrambler (v10.3), squared Euclidean distance was chosen to be the evaluating index of similarity.
4. Conclusion
A comprehensive and comparative analysis of five different species of snake sloughs was carried out applying a GC-MS based method. The results indicated that the chemical constituents varied greatly both in varieties and quantities. FAs (49.89%-54.65%) and steroids (13.55%-24.98%, mainly cholesterol) were the key compounds in all samples and the content of PUFA and cholesterol could be considered as the most important indexes to evaluate the medical value of snake sloughs. On the other hand, the GC-MS based method could be regarded as a referential technology for further snake slough related studies such as bioactivities, pharmacodynamics, pharmacokinetics, toxicology, etc.

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