

## Chemical Constituents of *Pedicularis longiflora* var. *tubiformis* (Orobanchaceae), a Common Hemiparasitic Medicinal Herb from the Qinghai Lake Basin, China

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**Abstract:** *Pedicularis longiflora* var. *tubiformis* (Orobanchaceae) is an abundant parasitic herb mainly found in the Xiaopohu wetland of the Qinghai Lake Basin in Northwestern China. The species has an important local medicinal value, and in this study, we evaluated the chemical profile of its stems, leaves and seeds using mass spectrometry. Dried samples of stems, leaves and seeds were grinded, weighted, and used for a series of extractions with an ultrasonic device at room temperature. The chemical profiles for each tissue were determined using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). Twenty-seven amino acids and organic acids were identified and quantified from stems, leaves and seeds. The content of amino acids detected in leaves and seeds was higher than the amount found in stems. Six flavonoids were also detected, including isoorientin, orientin, luteolin-7-*O*-glucoside, luteolin, apigenin and triclin. The concentrations of luteolin-7-*O*-glucoside, luteolin and triclin were the highest and more concentrated in leaves, while that of orientin was the lowest and mainly found in stems. Soluble monosaccharides and oligosaccharides below tetramer were also examined, and our analyses detected the presence of arabinose, fructose, galacturonic acid, glucose, glucuronic acid, inositol, sucrose, and trehalose. This is the first study to identify and quantify the main components of amino acids, organic acids, flavonoids and soluble sugars from stems, leaves and seeds of *P. longiflora* var. *tubiformis*. Eight of the amino acids detected are essential for humans, highlighting the medicinal importance of this species. Results shown here can be used as a reference case to develop future studies on the chemical constituents of *Pedicularis* herbs and other medicinal plants from the Tibetan region.



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**Keywords:** Amino acid content; flavonoids; gas chromatography-mass spectrometry (GC-MS); liquid chromatography-mass spectrometry (LC-MS); Qinghai-Tibet Plateau; Tibetan medicine

## 1 Introduction

*Pedicularis longiflora* Rudolph var. *tubiformis* (Klotzsch) Tsoong (Orobanchaceae) is a hemi-parasitic herb native to southwestern China (Qinghai, Xizang, Western Sichuan, Northwest Yunnan, and Southern Gansu) found in alpine meadows, marshes, lakes, valleys, streams, and spruce margins from 2700 to 5300 m. This plant is considered as one of the most important herbs used in the traditional medicine from Tibet, where it has been used to cool, hydrate and detoxify the body, strengthen tendons, and as a fixing essence [1,2].

In recent years, there have been several studies focused on identifying the chemical constituents and pharmacology of *P. longiflora* var. *tubiformis* [3–9]. The most relevant advances to date include the identification of four phenylpropanoid glycosides (echinacoside, pedicularioside A, pedicularioside M and verbascoside) using capillary electrophoresis [3]. In addition, both Zhang et al. [10] and Deng et al. [11] purified samples using a method involving silica-gel, macroporous resin, sephadex LH-20 and reversed phase C<sub>18</sub> columns, and identified seven chemical constituents (apigenin, chrysoeriol, isoverbascoside, luteolin, luteolin-4'-*O*- $\beta$ -D-glucoside, verbascoside, 3,5,7-trihydroxy-3', and 5'-dimethoxyl flavone) and 10 compounds (echinacoside, iridotrial glucoside, jionoside A1, kankanoside 1, kankanoside H1, luteolin, pedicularioside I,  $\beta$ -sitosterol, (*E*)-3-( $\beta$ -D-glucopyranosyloxy)-5-hydroxy-4'-methoxystilbene, and 5,7,4'-trihydroxy-3',5'-dimethoxyflavone) [10,11]. Ma et al. [5] reported 20 additional compounds, while Duo [7], Zhao et al. [8] and Ma et al. [9] found significant anticancer and antioxidation effects. In spite of all these findings, we still lack quantitative information on the chemical profiles and concentrations of the compounds present in different organs of this plant species.

The objective of this study was to identify and quantify the chemical constituents present in stems, leaves, and seeds of *P. longiflora* var. *tubiformis* using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). The use of mass spectrometry will allow us to compare the biochemical profiles from each tissue and test if there are differences in the compounds produced by different parts of the plant.

## 2 Materials and Methods

### 2.1 Plant Samples

Samples of *P. longiflora* var. *tubiformis* were collected from the Xiaopohu wetland (36°42'15"N, 100°47'07"E; 3210 m) in the Qinghai Lake Basin at Qinghai Province, China, where the species is locally abundant. The plants in this population had both open flowers and ripe fruits at the time of collection in July, 2018. A voucher specimen for the species was collected by X.S. (SX-2018-01) and deposited in the Herbarium of the Northwest Institute of Plateau Biology (HNWP) from the Chinese Academy of Sciences, Xining at the Qinghai Province.

### 2.2 Sample Preparation

Fresh samples of *P. longiflora* var. *tubiformis* were air-dried in shade for several days to preserve the integrity of their biochemical compounds. Then, stems, leaves and seeds were separated, grinded into powder, and stored at -20°C. To identify their chemical profiles, the three powdered tissues were treated under customized analytical procedures modified from previous studies, which are described below [5,10–14].

For the extraction of amino acids (AA) and organic acids (OA), 15 mg of frozen powder from each tissue was weighted and transferred into 2 ml microtubes containing 800  $\mu$ l of an 80% methanol-distilled water solution. After adding the internal standard "Norvaline", the extractions were performed with the

assistance of an ultrasonic device (TGCXZ-2B, Hongxianglong Technology Company, Beijing, China) at 4°C for about 1 h and centrifugated for 10 min at 12000 rpm. The supernatant was transferred into a 2 ml microtube, and a second extraction was conducted using the precipitate. The supernatants from the first and second extractions were combined, and its pH adjusted to 2.0 using 1 mol·L<sup>-1</sup> HCl. The combined supernatants were purified three times using an equal volume of diethyl ether-petroleum ether (1:1), and the soluble phase was retained. The soluble phase was then centrifugated and concentrated using a vacuum chamber, transferred to microtubes, dried with the vacuum chamber, and stored at -20°C [13].

To extract soluble sugars from samples, 15 mg of dried powder from the three tissues were weighted and transferred into 2 ml microtubes. Then, the extraction was performed using 800 µL of 80% methanol-distilled water in the ultrasonic device at room temperature for 1 h and preserved overnight at -4°C. The next day, residues were extracted again using 800 µL of 80% methanol-distilled water, and both extractions were combined and analyzed for soluble monosaccharides and oligosaccharides.

To isolate total flavonoids, 80 mg of each dried tissue powder was weighed and transferred into 2 ml microtubes with 1 ml methanol containing 0.1% ethylic acid. The extraction was performed using an ultrasonic device at 4°C for about 1 h, centrifuging samples for 10 min at 12000 rpm, and transferring the supernatant into 2 ml microtubes. This procedure was repeated a second time using the precipitate of the first extraction. Both supernatants were combined, centrifuged until dried, and resuspended in 400 µL of methanol. Finally, the experimental samples were filtered using microporous membranes (0.22 µm) and used for the mass spectrometry analyses [5,10,13,14]. The relative content of anthocyanins and flavonoids was calculated from peak areas of samples based on the intensity of the corresponding standard compounds, which include apigenin, cyanidin, cyanidin-3-rutinoside, isorientin, kaempferol, kaempferol-3-rutinoside, luteolin, luteolin-7-O-D-glucopyranoside, orientin, pelargonidin, pelargonidin-3,5-diglucoside, proanthocyanidin B1, proanthocyanidins, quercetin, quercetin-3-glucoside, rutin, scopoletin, tricetin and verbascoside. For compounds lacking corresponding standards, their quantification was carried out using similar compounds [15].

### 2.3 Gas Chromatography-Mass Spectrometry Analysis

The extractions of AA, OA, and soluble sugars described above were evaporated until completely dried and treated with 50 µL (20 mg/mL) of methoxylamine hydrochloride (Sigma-Aldrich, Saint Louis, Missouri, USA) in anhydrous pyridine at 30°C for 1.5 h, and then with 80 µL of N-methyl-N-(trimethylsilyl)-rifluoroacetamide at 37°C for 30 min.

Samples to be analyzed for AA and OA content were dissolved in hexane before the procedure, while samples for soluble sugar components were dissolved in 200 µl of EtOAc and hexane (1:1). All samples were processed in a gas chromatography-mass spectrometer (GC-QqQ MS 7890/7000C, Agilent Technologies, Santa Clara, California, USA) with a fused silica glass capillary (Agilent J&W DB-5 column, 30 m × 250 µm × 0.1 µm film thickness, Agilent Technologies, Santa Clara, California, USA). Injection and interface temperatures were 260°C and 280°C, respectively. The column temperature gradient was maintained at 80°C for 5 min; 80–210°C for 22 min; 210–300°C for 1 min; and at 300°C for 5 min. The settings used in the GC-MS equipment for the analyses were ion source of EI and scan mode of TIC.

### 2.4 Liquid Chromatography-Mass Spectrometry Analysis

For the identification and quantification of flavonoids, 10 mg of frozen powder from each tissue was treated with a solution of 1 ml 0.1% acetic acid-methanol at 4°C overnight, and then centrifuged for 10 min at 10000 rpm. The supernatant was collected and dried using a vacuum centrifuge concentrator (CV100-DNA, Aijimu, Beijing, China) and stored at -20°C. The dried extracts were resuspended in MeOH immediately before being analyzed with an ultra-high-performance liquid chromatography-mass spectrometry (UPLC-MS/MS, Milford, MA, USA) coupled to a triple-Quadrupole Mass Spectrometry

(XEVO®-TQ) with electrospray ionization (ESI). The separation was carried out with a ZORBAX Eclipse plus C<sub>18</sub> (150 mm × 3.0 mm; Agilent Technologies, Santa Clara, California, USA) with a particle size of 1.8 μm at 40°C. The gradient was prepared using 0.1% formic acid (A) and acetonitrile (B) as the mobile phases, with time intervals of 0–1 min (5% B), 1–8 min (5–30% B), 8–12 min (40–95% B), 16–17 min (95–100% B), 17–21 min (100% B), and 21–25 min (5% B). The operating conditions included a flow rate of 1.0 ml·min<sup>-1</sup>, positive ion ESI modes, capillary voltages at 3.0 kV, and 16 L·h<sup>-1</sup> of nebulization nitrogen flow.

### 3 Results

Our analyses detected 27 AA and OA present in the stems, leaves and seeds of *P. longiflora* var. *tubiformis*. The highest concentrations of AA were found on leaves, while the lowest were detected on stems. The three AA with higher concentration on leaves were L-asparagine (2933.69 ± 462.41 μg/g), valine (307.93 ± 30.40 μg/g) and L-alanine (285.44 ± 16.23 μg/g), while the lowest were glycine (19.97 ± 0.50 μg/g), cysteine (13.80 ± 1.04 μg/g) and methionine (13.57 ± 0.31 μg/g) (Tab. 1). Regarding seeds, the most common AA were L-asparagine (3108.85 ± 273.93 μg/g), glutamic (2528.78 ± 167.34 μg/g) and aspartic (1352.68 ± 41.21 μg/g), whereas the lowest were glycine (21.36 ± 1.41 μg/g), methionine (13.32 ± 0.28 μg/g) and cysteine (10.26 ± 0.27 μg/g), following a similar pattern similar to the one observed for leaves (Tab. 1). Data for the AA content in stems showed that the three most abundant compounds were arginine (27.92 ± 0.29 μg/g), tyrosine (21.38 ± 0.17 μg/g) and histidine (18.91 ± 0.19 μg/g), while the

**Table 1:** Comparison of the content of 19 amino acids in the stems, leaves and seeds of *Pedicularis longiflora* var. *tubiformis*

Tissue	Stems (μg/g)	Leaves (μg/g)	Seeds (μg/g)
L-alanine	0.76 ± 0.06c	285.44 ± 16.23a	223.49 ± 8.94b
Glycine	3.65 ± 0.31b	19.97 ± 0.50ab	21.36 ± 1.41a
β-alanine	5.72 ± 0.14b	171.35 ± 21.41a	148.03 ± 4.68ab
Valine	9.00 ± 0.18c	307.93 ± 30.40a	202.35 ± 5.99b
Leucine	6.28 ± 0.13c	61.80 ± 3.27a	41.97 ± 1.26b
Isoleucine	10.54 ± 0.12c	214.87 ± 9.93a	43.49 ± 1.30b
Proline	5.69 ± 0.14c	55.18 ± 5.10a	44.81 ± 1.03b
Methionine	12.13 ± 0.12a	13.57 ± 0.31a	13.32 ± 0.28a
Serine	13.60 ± 0.65c	204.57 ± 6.84a	80.87 ± 1.98b
Threonine	12.61 ± 0.13c	88.17 ± 1.75a	65.06 ± 0.67b
Phenylalanine	15.40 ± 0.12c	120.96 ± 3.02a	32.14 ± 0.30b
Aspartic	8.12 ± 0.03c	138.24 ± 10.21b	1352.68 ± 41.21a
Cysteine	3.07 ± 0.03c	13.80 ± 1.04a	10.26 ± 0.27b
Glutamic	12.52 ± 0.29c	157.05 ± 12.03b	2528.78 ± 167.34a
L-asparagine	12.75 ± 0.24b	2933.69 ± 462.41a	3108.85 ± 273.93a
Lysine	9.68 ± 0.17c	65.82 ± 2.77a	33.64 ± 1.93b
Arginine	27.92 ± 0.29c	162.02 ± 18.86b	621.50 ± 23.03a
Histidine	18.91 ± 0.19c	29.51 ± 4.20b	48.68 ± 2.98a
Tyrosine	21.38 ± 0.17c	61.74 ± 1.88a	48.93 ± 1.88b

Data is expressed following the format "Mean ± Standard Deviation". Small-case letters along same columns indicate significant differences among groups ( $p < 0.05$ ; one-way ANOVA).

three less represented were glycine ( $3.65 \pm 0.31 \mu\text{g/g}$ ), cysteine ( $3.07 \pm 0.03 \mu\text{g/g}$ ) and L-alanine ( $0.76 \pm 0.06 \mu\text{g/g}$ ) (Tab. 1). As for OA in stems, the highest concentrations measured were for fumaric acid ( $24674.50 \pm 795.46 \mu\text{g/g}$ ), malic acid ( $5467.64 \pm 339.42 \mu\text{g/g}$ ) and citric acid ( $4188.51 \pm 158.42 \mu\text{g/g}$ ), while the lowest were GABA ( $0 \mu\text{g/g}$ ) and L-pyroglutamic acid ( $13.80 \pm 0.42 \mu\text{g/g}$ ). In leaves, our results indicate that the OA with highest concentrations detected were again fumaric acid ( $6136.05 \pm 553.03 \mu\text{g/g}$ ), malic acid ( $2877.79 \pm 269.49 \mu\text{g/g}$ ) and citric acid ( $1658.29 \pm 179.29 \mu\text{g/g}$ ), while the lowest were oxalic acid ( $116.59 \pm 0.83 \mu\text{g/g}$ ), GABA ( $120.54 \pm 5.75 \mu\text{g/g}$ ) and succinic acid ( $94.87 \pm 2.62 \mu\text{g/g}$ ). Last, our measurements of OA in seeds indicate that the malic acid ( $1604.00 \pm 158.86 \mu\text{g/g}$ ) and citric acid ( $1092.31 \pm 81.33 \mu\text{g/g}$ ) were the most concentrated compounds detected, while the lowest were oxalic acid ( $45.68 \pm 3.25 \mu\text{g/g}$ ) and GABA ( $26.50 \pm 2.97 \mu\text{g/g}$ ) (Tab. 2).

**Table 2:** Comparison of the content of eight organic acids related to amino acids in the stems, leaves and seeds of *Pedicularis longiflora* var. *tubiformis*

Tissue	Citric acid	Fumaric acid	GABA	L-Pyroglutamic acid	Malic acid	Oxalic acid	Oxaloacetic acid	Succinic acid
Stems ( $\mu\text{g/g}$ )	$4188.51 \pm 158.42\text{a}$	$24674.50 \pm 795.46\text{a}$	0	$13.80 \pm 0.42\text{c}$	$5467.64 \pm 339.42\text{a}$	$77.97 \pm 2.17\text{b}$	$317.65 \pm 24.73\text{b}$	$362.40 \pm 17.81\text{a}$
Leaves ( $\mu\text{g/g}$ )	$1658.29 \pm 179.29\text{b}$	$6136.05 \pm 553.03\text{b}$	$120.54 \pm 5.75\text{a}$	$777.59 \pm 29.62\text{a}$	$2877.79 \pm 269.49\text{b}$	$116.59 \pm 0.83\text{a}$	$729.66 \pm 29.93\text{a}$	$94.87 \pm 2.62\text{b}$
Seeds ( $\mu\text{g/g}$ )	$1092.31 \pm 81.33\text{c}$	$416.52 \pm 33.52\text{c}$	$26.50 \pm 2.97\text{b}$	$237.91 \pm 11.79\text{b}$	$1604.00 \pm 158.86\text{c}$	$45.68 \pm 3.25\text{c}$	$202.12 \pm 10.70\text{c}$	$50.97 \pm 5.32\text{c}$

Data is expressed following the format "Mean  $\pm$  Standard Deviation". Small-case letters along same columns indicate significant differences among groups ( $p < 0.05$ ; one-way ANOVA).

In the case of flavonoids, six compounds were detected (Tab. 3). The most concentrated flavonoids found in stems were triclin ( $10.18 \mu\text{g/g}$ ) and luteolin ( $8.85 \mu\text{g/g}$ ), while luteolin-7-*O*-glucoside ( $1699.62 \mu\text{g/g}$ ), isorientin ( $2.82 \mu\text{g/g}$ ), luteolin ( $487.74 \mu\text{g/g}$ ), apigenin ( $188.01 \mu\text{g/g}$ ) and triclin ( $299.67 \mu\text{g/g}$ ) showed high levels in leaves. It is interesting that we did not detect traces of orientin in leaves (Tab. 3). In seeds, the highest concentrations of flavonoids were for triclin ( $41.39 \mu\text{g/g}$ ) and apigenin ( $26.29 \mu\text{g/g}$ ), while the lowest levels were for orientin ( $0.13 \mu\text{g/g}$ ) (Tab. 3).

**Table 3:** Comparison of the flavonoid contents in the stems, leaves and seeds of *Pedicularis longiflora* var. *tubiformis*

Peak No.	Retention time/min	$\lambda_{\text{max}}/\text{nm}$	Parent ion	$\text{MS}^2$	Identification name	Stems ( $\mu\text{g/g}$ )	Leaves ( $\mu\text{g/g}$ )	Seeds ( $\mu\text{g/g}$ )	Reference
1	7.64	347.8, 268.8	449.3	–	Isoorientin	0.75	2.82	0.81	Standards
2	7.94	347.8, 255.8	449.3	–	Orientin	0.49	Undetected	0.13	Standards
3	8.74	347.8, 253.8	449.2	287.2	Luteolin-7- <i>O</i> -glucoside	5.33	1699.62	22.30	Standards
4	12.18	347.8, 248.8	287.2	–	Luteolin	8.85	487.74	17.87	Standards
5	13.85	336.8, 266.8	271.2	–	Apigenin	2.36	188.01	26.29	Standards
6	14.02	347.8, 245.8	331.3	–	Triclin	10.18	299.67	41.39	Standards

About the types of monosaccharides and oligosaccharides present per tissue type, a multiple comparative analysis detected significant differences in the content of eight of them (arabitol, fructose, galacturonic acid, glucose, glucuronic acid, inositol, sucrose, and trehalose) among stems, leaves and seeds, except for fructose ( $p < 0.05$ ; Tab. 4). For instance, the concentration of glucose was the highest in stems and leaves with  $6.7993 \pm 0.1296 \text{ mg/g}$  and  $8.5927 \pm 0.4144 \text{ mg/g}$ , respectively, and contrasting with sucrose ( $25.5663 \pm 1.3915 \text{ mg/g}$ ), which was the highest in seeds (Tab. 4). The monosaccharides

**Table 4:** Comparison of the content of eight soluble monosaccharides and oligosaccharides in the stems, leaves and seeds of *Pedicularis longiflora* var. *tubiformis*

Tissue	Arabitol	Fructose	Galacturonic acid	Glucose	Glucuronic acid	Inositol	Sucrose	Trehalose
Stems (mg/g)	0.6580 ± 0.0648b	0.7603 ± 0.0116a	0.1023 ± 0.0020a	6.7993 ± 0.1296b	0.0440 ± 0.0015b	0.1100 ± 0.0069b	0.1937 ± 0.0091c	0.1953 ± 0.0145b
Leaves (mg/g)	1.3323 ± 0.0482a	0.7450 ± 0.0431ab	0.0050 ± 0.0006c	8.5927 ± 0.4144a	0.1363 ± 0.0067a	1.0383 ± 0.0500a	2.3117 ± 0.2631b	0.5910 ± 0.0595a
Seeds (mg/g)	0.2263 ± 0.0069c	0.0733 ± 0.0013b	0.0127 ± 0.0003b	0.4697 ± 0.0320c	0.0133 ± 0.0009c	0	25.5663 ± 1.3915a	0.0767 ± 0.0009c

Data is expressed following the format "Mean ± Standard Deviation". Small-case letters along same columns indicate significant differences among groups ( $p < 0.05$ ; one-way ANOVA).

and oligosaccharides with lowest concentrations were glucuronic acid in seeds ( $0.0133 \pm 0.0009$  mg/g) and galacturonic acid ( $0.0050 \pm 0.0006$  mg/g) in leaves, while inositol was not even detected in seeds.

#### 4 Discussion

The chemical profile reported here for AA, OA, flavonoids, and soluble sugars in *P. longiflora* var. *tubiformis* is congruent with the findings reported by previous authors when examined whole plants. In contrast to previous studies, we have dissected the concentrations and biochemical compounds per tissue, providing a valuable reference for future studies on the organs with the most medicinal value in this species. A recent study conducted by Deng et al. [11] on the chemical constituents of a population of *P. longiflora* var. *tubiformis* from Deqin County (Yunnan Province, ca. 900 km SW from our sampling site) found that the content of luteolin there was  $12.55 \mu\text{g/g}$ , while the content of tricetin was  $9.85 \mu\text{g/g}$ , which is close to the values we obtained from stems [11]. Zhang et al. [10] found that the population of the same taxon from Gangcha County, Qinghai Province (in the Qinghai Basin, within 100 km from our sampling site) showed  $31 \mu\text{g/g}$  of luteolin and  $20.5 \mu\text{g/g}$  of apigenin, which resemble the values we obtained from seeds (Tab. 3) [10]. In the Huzhu Northern Mountain National Forest Park at Qinghai (ca. 100 km NE from our sampling site), Ma et al. [5] reported  $10.67 \mu\text{g/g}$  of luteolin,  $8.33 \mu\text{g/g}$  of apigenin, and  $8 \mu\text{g/g}$  of tricetin. These values are similar to the data we obtained for stems, whose concentrations were considerably higher in our sampling (Tab. 3).

In spite of having a substantial number of earlier studies exploring the chemical constituents of *P. longiflora* var. *tubiformis*, none of them measured the compounds and concentrations present in individual parts of the plant. Therefore, we cannot draw effective comparisons between our results and the ones presented in previous studies. Regarding the discrepancies observed in the type and concentrations of AA identified in each of the three tissues investigated, we speculate that these might be related to growth stages and metabolic activities of sampled plants, environmental and/or genetic factors [16]. For example, we found that the content of flavonoids in leaves was relatively higher than the one measured in seeds and stems (except for orientin). A possible explanation for this trend is that concentrations are expected to be higher in leaves than in stems and seeds because over 2% of the carbon fixed during photosynthesis is eventually converted into flavonoids [17]. In humans, flavonoids have antioxidant effects which eliminate free radicals in the body [18]. For instance, luteolins can inhibit the proliferation of cancer cells, resist inflammation and oxidation, and reduce the damage caused by excessive reactive oxygen [19].

The differences found in the chemical constituents present among stems, leaves and seeds of *P. longiflora* var. *tubiformis* have complex mechanisms for regulation, and further research is needed on the specifics on how these processes operate. Interestingly, the chemical compounds identified and quantified in this study have been reported with biological activities in previous chemical surveys [20–24]. In fact, a number of the constituents identified we report for *P. longiflora* var. *tubiformis* have important medical applications such as the OA GABA, which can be used as an anticancer drug; the flavonoid luteolin-7-*O*-glucoside, that can increase anti-oxidant and anti-inflammatory activities and attenuate the side effects of

anticancer drugs; and the soluble sugar D-chiro-inositol, reported to improve insulin resistance and menstrual cycle in women with polycystic ovary syndrome [20–24].

## 5 Conclusions

In conclusion, we identified and quantified 27 AA and OA, and six flavonoids and eight soluble sugars from the stems, leaves, and seeds of *P. longiflora* var. *tubiformis* using GC-MS and LC-MS techniques. To our knowledge, this is the first study exploring the chemical profiles per tissue using mass spectrometry in this species. Eight of the AA detected here are essential for humans, highlighting the nutritional importance of this plant and the need to explore in more depth its medicinal properties. This study can also serve as a reference for further studies on the bioactive compounds of *Pedicularis* herbs, and to design future studies applying GC-MS and LC-MS to other important medicinal plants used in the traditional Tibetan medicine.

**Author Contribution:** Feng Liu and Zilan Ma made the experiment and analyzed the experimental data. Xu Su and Yuping Liu designed this research as well as drafted the manuscript. Marcos A. Caraballo-Ortiz and Hui Zhang polished the manuscript.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

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