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Review

Chromatographic and electrophoretic methods for pharmaceutically active compounds in *Rhododendron dauricum*

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Abstract

In this review, chemical constituents present in *Rhododendron dauricum* L. were briefly surveyed, and the methods of pretreatment of this plant prior to analysis were also summarized. The analysis methods reported for determining pharmaceutically active compounds in *R. dauricum* L. include gas chromatography with mass spectrscopy, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). In addition, both advantages and disadvantages of the above methods were mentioned.
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Keywords: Reviews; *Rhododendron dauricum*

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1. Introduction

Rhododendron dauricum L., referred as 'Man-shan-hong' in Chinese, belongs to the family of Ericaceae, the genera of *Rhododendron* L. [1]. *R. dauricum* L. is distributed in the northern part of China, eastern part of Siberia, and Hokkaido in Japan. This plant blooms in the spring, and the purple flowers possess flamboyant color, as well as fragrance. So, *R. dauricum* L. is not only a valuable ornamental, but the essential oil from its flowers is a kind of rare flavor, used in cosmetic, food and beverage [2]. Moreover, the leaves and roots of *R. dauricum* L. are important crude drugs in traditional Chinese medicines. The dried leaves of this plant are used medicinally

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as an expectorant and to treat acute-chronic bronchitis, and the roots are used for therapy of acute bacteroidal dysentery [3].

Dried leaves of *R. dauricum* L. were found accidentally to have functions of relieving a cough, removing the phlegm and suppressing the asthma in 1959, when people tried to treat Keshan disease in China [4]. With this plant, more than 1800 patients suffering chronic bronchitis had been cured in 1965 and in 1966. Since then, the chemical components, pharmacological properties and clinical curative effect of the herb have been investigated. Chinese Pharmacopoeia (1977 edition) listed *R. dauricum* L. as an official drug. Nowdays, *R. dauricum* L. and its preparations such as Qin-Bao-Hong capsules are employed clinically, and its phytopharmacuticals are readily available commercially. Recently, the methanol extract of the leaves and twigs of *R. dauricum* L. was found to display significant anti-HIV activity (EC_{50} ≤20 μg/mL, TI >5) [5].

2. Chemical constituents and pharmacological properties

Up to now, about 90 pharmaceutically active molecules have been identified in *R. dauricum* L. These active ingredients may be classified into five groups: flavonoids, phenolic acids, terpenoids, coumarins and volatile oils.

2.1. Flavonoids

So far, there are numerous flavonoids in the leaves and twigs of *R. dauricum* L. have been reported. They are mainly composed of flavonone, flavonol, flavone and flavonol glucoside. These flavonoids include ferrerol, hyperoside, kaemferol, quercetin, azaleatin, gossypetin, myricetin, dihydro-quercetin, dihydro-kaemferol, dihydro-myricetin, avicularin, 8-desmethlyfeaaerol, metteucinol, isohyperoside, 5-methyl kaemferol and 5-methyl myricetin [6–10], and their molecular structures were shown in Fig. 1. Recently, Dai et al. [11] isolated and identified eight compounds including quercetin, isorhamnetin, hyperoside, cacticin, hirsutine, kaemferol-3-β-D-galactoside, myricetin-3-β-D-xyloside and 6′′-O-(4-hydroxybenzoyl) hyperoside. The total contents of flavonoids are in the range of 2.6–4.0%, which varies with the season of harvest and the contents of flavonoids are maximum when herborized in October [12]. Some related investigations showed that flavonoids have a broad range of physiological activities such as anti-inflammatory [13], anti-bacterial [14] and antioxidant activity for scavenging radicals [15,16] and inhibition of a variety of enzymes [17]. Pathological experiments have demonstrated that farrerol and 8-desmethlyfarrerol have significant ability to move the phlegm [18]. Farrerol has been synthesized chemically as an antibechic [19]. Hyperoside and isohyperoside can alleviate a cough [20]. Quercetin, as a major active component of this plant, can prevent LPS-induced tumor necrosis factor-α and/or NO overproduction, which result in many immunomodulatory, infectious and inflammatory diseases such as sepsis syndrome, bacterial meningitis, cerebral malaria, adult respiratory distress syndrome, AIDS and rheumatoid arthritis [21,22]. It can also inhibit capillary permeability and intestinal movement in the abdominal cavity, which leads to antidiarrheal activity [23]. Aavicularin and hyperoside have antinociceptive action [24].

2.2. Phenols

The leaves of *R. dauricum* L. contain many kinds of phenols. The major phenols are phenolic acids, accompanied by hydroquinone [25], 4-*o*-methylphloroacetophenone [26], and arbutin [27].

The phenolic acids include *p*-hydroxybenoic acid, protocatechuic acid, syringic acid, vanillic acid, gallic acid 3-monomethyl ether and anisic acid [28], and their molecular structures were shown in Fig. 2. These compounds were reported to have antibacterial, anti-fungal, anti-inflammatory, anti-oxidant and styptic activities [29]. The new phenolic acids, two isomeric rhododaurichromanic acids A and B [5], together with the chromene daurichromenic acid [30] have been isolated from *R. dauricum* L., and their structures were shown in Fig. 3. Daurichromenic acid was demonstrated to have potent anti-HIV activity with an EC_{50} value of 0.00567 mg/mL and therapeutic index (TI) of 3.710. Rhododaurichromanic acid A also shows relatively potent anti-HIV activity with an EC_{50} value of 0.37 mg/mL, and a TI of 91.9, whereas rhododaurichromanic acid B displays no anti-HIV activity.

4-*o*-Methylphloroacetophenone, shown in Fig. 4, displays strong activity against all fungal species [31]. Arbutin has an inhibitory effect on tyrosinase activity, and is extensively used as a kind of skin-lightening agent in cosmetics [32]. It has been discovered in *R. dauricum* L., and its content is between 10.66 and 13.66% depending on the determination methods.

2.3. Triterpenoids

Treterpenoids in *R. dauricum* L. mainly consist of oleanolic acid [33] and andromedotoxin [5], and their molecular structures are shown in Fig. 5. Oleanolic acid has anti-inflammatory, antihyperlipidemic and antitumor-promotion effects, and protects liver against chemically induced injury [34]. Its content in *R. dauricum* L. ranges from 0.33 to 0.66%, varying with the place of production and the season of harvest. Oleanolic acid is relatively non-toxic, and has been used in cosmetics and health products. Contrarily, andromedotoxin is a kind of toxin, which exerts toxic effects by binding to sodium channels in cell membranes and increasing the permeability of sodium ions in excitable membranes [35]. On the other hand, andromedotoxin has abirritation [36], and its abirritation

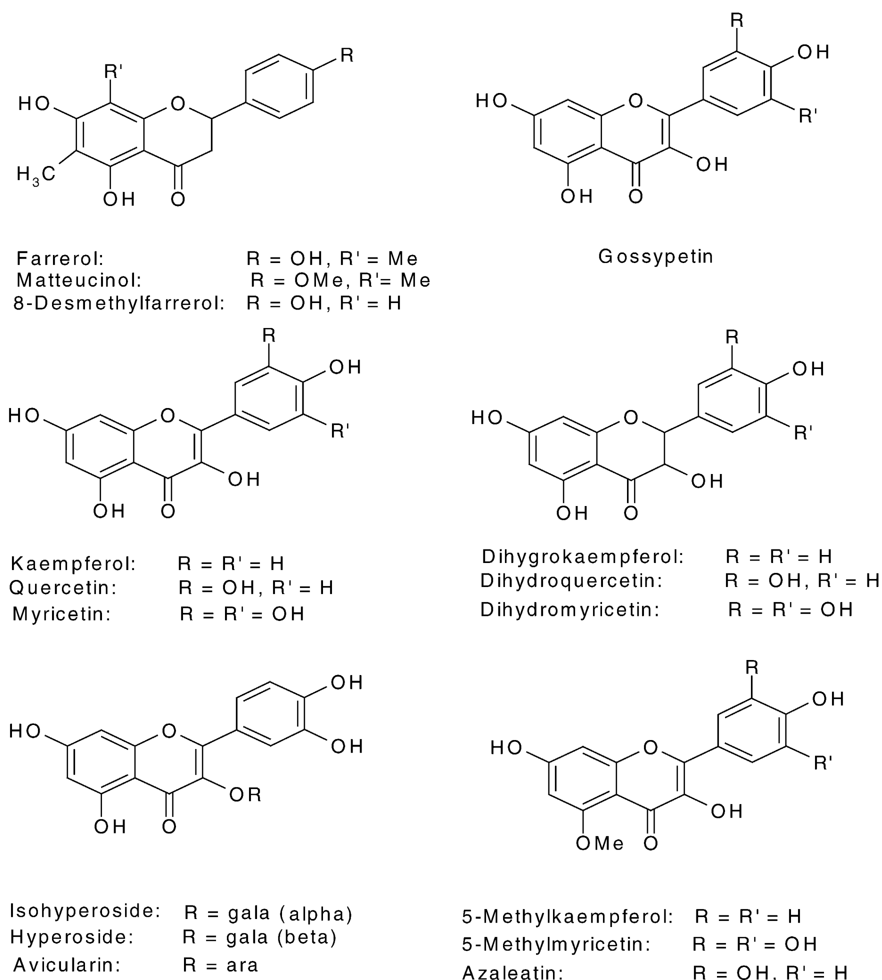


Fig. 1. Molecular structures of the flavonoids in *R. dauricum* L.

index is more than 8.60. Its content in this plant is about 0.003%.

2.4. Coumarins

Umbelliferone and scopoletin [5] are found in *R. dauricum* L., their molecular structures are shown in Fig. 6. These coumarins have significant biological properties such as anti-inflammatory, antioxidant and antiplatelet aggregation activity [37].

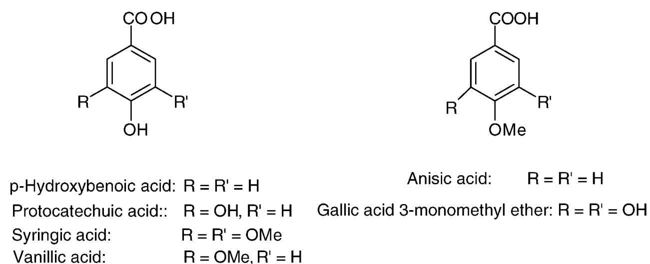


Fig. 2. Molecular structures of the phenolic acids in *R. dauricum* L.

2.5. Volatile oils

The leaves of *R. dauricum* L. contain volatile compounds for treatment of cough and asthma. About 39 constituents of the volatile oils [38,39] have been identified. These compounds include 12 terpenes, 16 sesquiterpenes, and some other compounds such as alcohols, ketones and esters. The total content of essential oils is about 3.2%. In general, germacrone in essential oil of *R. dauricum* L. is recognized as the pharmaceutically active component to treat asthma.

Twenty-three volatile compounds from buds of *R. dauricum* L. have been identified, and the total content

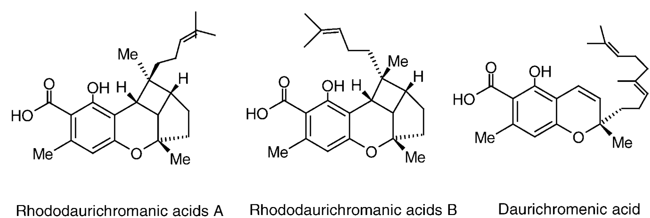
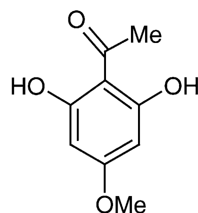


Fig. 3. Molecular structures of rhododaurichromanic acid A, rhododaurichromanic acid B and daurichromenic acid.



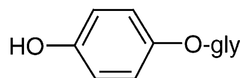
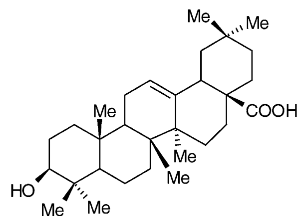


Fig. 4. Molecular structures of 4-*o*-methylphloroacetophenone and arbutin.



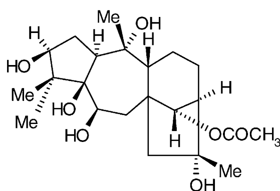
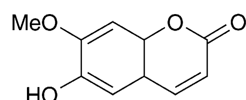


Fig. 5. Molecular structures of terpenoids in *R. dauricum* L.

is about 0.27%. They are also composed of terpenes and sesquiterpenes, which have significant biological properties. However, the flowers of *R. dauricum* L. have not been explored for pharmaceutical purposes.

3. Chromatographic and electrophoretic methods

Crude herbs are always composed of numerous compounds; analysis of active ingredients in *R. dauricum* L. is a challenging task. Chromatographic and electrophoretic methods are the preferred analytical techniques because of their high separation abilities prior to detection of individual components. These methods allow the determination of analytes in the presence of coexisting compounds, and avoid interference. The choice of either chromatographic or electrophoretic method to use depends on the analyte property, the sensitivity required, the complexity of the sample matrix, the time spent for sample pretreatment, the chromatographic resolution required, and the expense. Several techniques available for the determination of active constituents in *R. dauricum* L. are discussed in the following sections.



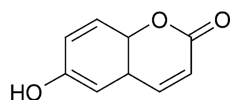


Fig. 6. Molecular structures of coumarins in *R. dauricum* L.

3.1. Gas chromatography–mass spectrometry (GC–MS)

GC–MS [40] is the most important analytical technique for volatile substances, because it provides simultaneous separation, identification, confirmation and quantitation. In particular, for the low concentrations of unknown volatile oils in leaves and flowers of *R. dauricum* L., GC–MS is the mainstay of the analysis of these compounds [41]. A simple procedure for the isolation of volatile oil from sample matrix was based on distillation through water vapor for several hours, and followed by extraction with anhydrous ether. After removal of solvent by evaporation, the volatile oils are obtained and analyzed by GC–MS. Another pretreatment method is simultaneous distillation and solvent extraction. Nickerson and Likens [42] put forward this method in 1966, allowing distillation and solvent extraction together in one step. This method is often applied to extract volatile oils in crude herbs.

The technique of simultaneous distillation and solvent extraction could isolate volatiles in mg/L magnitude from polar or non-polar matrices, and concentrate these volatiles thousandfold. Furthermore, with a simultaneous distillation and solvent extraction method, volatile oils are dissolved in the solvent, and are easily obtained later by solvent evaporation. Whereas in a distillation method, volatile oil may be adhered to distilling tube or other recipient, resulting in the loss of volatile oils and difficulty of transfer. Li et al. [43] have used a GC–MS method for the determination of volatile oils from leaves of *R. dauricum* L., collected in Liang-shan (Si-chuan, China). With simultaneous distillation and solvent extraction, volatile oils were exacted and concentrated prior to GC–MS analysis. The chromatographic conditions were: capillary column HP5 (0.25 mm × 30 m × 0.25 μm), operation temperature in the range of 30–240 °C, at 15 °C/min, split injection with 75:1 split ratio, mass selective detector, injection temperature 250 °C, helium was used as carrier gas. Using these chromatographic conditions, 72 peaks were separated, of which 29 compounds were identified and quantified. The chromatogram and analysis results are shown in Fig. 7 and Table 1, respectively. The main components are olefins (32.53%), saturated hydrocarbons (10.13%), alcohols (6.26%) and esters (15.29%), etc. Among these compounds, the contents of α-pinene, tetradecane, α-terpineol, hexanoic acid methyl ester are the highest. Xin et al. [44] have applied GC–MS to analyze volatile oils from flowers of *R. dauricum* L., collected in Hei-long-jiang arboretum (Hei-long-jiang, China). With stream distillation, yellowish and sweet-smelling oils were prepared, and the yield was about 0.4%. The oils were analyzed by GC–MS, and 39 compounds were identified and quantitated, which contributed 96.66% of the volatile oils. The principle constituents include terpenes (17.61%), and sesquiterpenes (63.65%), and (−)-borneol acetate (10.62%), (*Z*,*E*)-α-farnesene (24.29%) and α-bisabolene (12.28%) are the major componets. With GC–MS, Zhang et al. [45] determined volatile oils from buds of *R. dauricum* L., collected in Qianshan (Liao-ning, China). Hundred grams of buds were distillated for 4 h, and then distillates

Table 1
The constituents of volatile oil in leaves of *R. dauricum* L.

Peak	Migration time (min)	Compound	Content (%)
3	4.515	α-Pinene	9.982
6	5.014	Bicyclo[3.1.0]hexane, 4-m4thlene-1-(1-methylethyl)-	3.79
10	5.602	Bicyclo[5.1.0]octane, 8-isopropylidene	7.157
13	5.972	3-Chloro-1-heptene	2.157
14	6.017	3-Chloro-1-heptene	2.665
15	6.095	3-Octane, 6-methyl	1.754
25	7.452	3-Cyclohexene-1-methyl, α,α,4-trimethyl	3.723
26	7.754	Hexanoic acid, methyl ester	3.232
31	9.312	β-Copaene	0.356
33	9.402	Tetradecane	8.98
35	9.705	α-Santalene	0.341
36	10.761	Caryophylene	0.98
40	10.243	α-Copaene	2.06
42	10.423	Isolongifolene	1.05
43	10.456	Aromadendrene	2.10
44	10.591	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-dimethyl-4-methylene-1-(1-methylethyl-)	0.700
45	10.659	d-δ-Cadinene	2.00
47	10.861	1,3,3-Dioxabarinane, 4,6-dimethyl-2-(1-methylbutoxy-)	0.703
48	10.928	1,6,10-Dodecatrien, 3,7,11-trimethyl	0.715
49	11.152	Hexadecane	0.567
57	11.713	Mayurone	0.755
59	11.994	α-Bisabolol	1.848
60	12.297	2(1H)-naphthalenonee, 4a,5,8,8a-tetrahydro-1,1,4a-trimethyl-	1.638
65	13.105	*N*, *N*-butytrylpyrrolidine	0.339
66	13.217	Acetamide, *N*-methyl-*N*-[4-[4-methoxy 1-hexahydropyridyl]-2-butynl]-	0.964
67	13.351	Dibutyl phthalate	4.598
71	14.035	1,2-Benzenedicarboxylic acid, butyl-2-methylpropyl ester	7.455
72	15.110	Phytol	0.635

Source: Li et al. [42].

were extracted with anhydrous ether. After dehydration with anhydrous sodium sulfate, and solvent removal by evaporation, a colorless oil was gained, the yield was about 0.27%. Twenty-three compounds were separated and identified. The majority of constituents in the volatile oil are borneol acetate (36.64%), caryophylene (18.26%) and β-camphene (3.31%).

From the above two experiments, it was concluded that the composition of volatile oils in buds of *R. dauricum* L. is greatly different from that in flower. It is also noticeable that the content and composition of volatile oils varies with collection time.

Furthermore, several other papers reported that GC–MS has been used to assay the votalile oils of *Rhododendron thymifolium Maxim*. [46], *Folium Rhododendri daurici* [47], *Rhododendron* Spp. [48] and *R. Maxim*. [49].

3.2. Thin layer chromatography (TLC)

TLC continues to be widely used for the standardization of plant materials used as traditional medicines [50], because it is suitable for the analysis of complex and dirty samples with poor detection characteristics, for stability and content uniformity testing. The Chinese Pharmacopoeia (1977 version) recommended TLC to identify *R. dauricum* L. and farrerol as a marker substance to evaluate the quality.

Zhong et al. [51] used TLC method to control the quality of *R. dauricum* L. To avoid the influence of chlorophyll, sample preparation and TLC conditions were optimized. Dried leaf samples were extracted with ether. After filtration and evaporation of the ether, residues were dissolved with 40% ethanol. Then after filtration, evaporation of solvent, re-dissolving procedures were repeated again, and the residues were eventually dissolved in methanol. Removal of chlorophyll was based on the solubility difference between farrerol and chlorophyll in ethanol. The plates were coated with Silica Gel G containing 3% CMC-Na. The solvent system was toluene/ethyl acetate/formic acid (7:2:0.5). The plates were developed, dried and then sprayed with a solution of aluminum chloride. The plates were located under ultraviolet light (365 nm) to inspect spots. Correspondingly, standard solution of farrerol was analyzed in the same procedures, and the similar color spots should be located in the same position as in sample analysis. Compared with the method recommended by Chinese pharmacopoeia, this approach is simpler and more effective to separate and identify *R. dauricum* L.

Since all sample components are located in the chromatogram, TLC is the most suitable for surveying sample properties; hence it is increasingly used to determine botanical origin, classification fractionation and speciation of plant material. The *Rhododendron* species consists of about 850 species, and it is difficult to survey without some subdivision. Several classifications have been proposed such as randomly amplified polymorphic DNA (RAPD) [52] and chemotaxonomy [53]. Hu and Xiao [54] have used HPTLC for

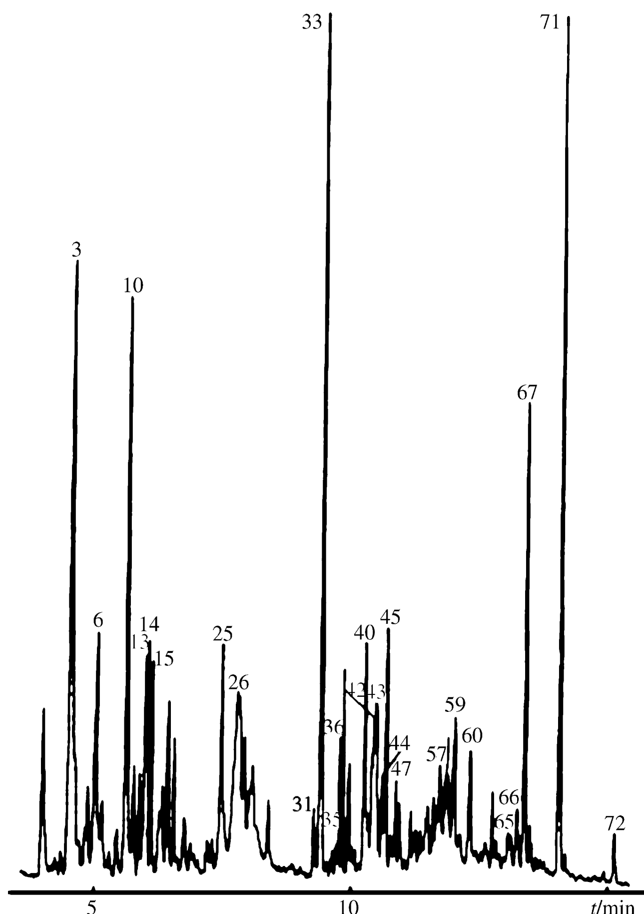


Fig. 7. GC chromatogram of the volatile oils in leaves of *R. dauricum* L. (figure from Li et al. [42]). Capillary column HP5 (0.25 mm × 30 m × 0.25 μm); operation temperature: in the range of 30–240 °C, at 15 °C/min; split injection, split ratio 75:1; HP5791 mass selective detector; injection temperature: 250 °C; carrier gas: helium. Peak identifications are the same as in Table 1.

chemotaxonomic study of *Rhododendron*. One hundred and seventy-seven samples of the genus, including *R. dauricum* L., were investigated by analysis of 16 flavonoids and three other phenols with HPTLC silica gel plate-wise development and polyamide thin layer. Owing to diversity of components in leaves of *Rhododendron* species and their great difference in polarity, plate-wise development method was adopted for separation as completely as possible. The sample solution and a standard mixture solution were simultaneously spotted by means of a micropipette in one plate of silica gel. It was first developed with petroleum ether (boiling point 60–90 °C)/ether/formic acid (62:31:7), and matteucinol, farrerol, kaempferide, kaempferol, umbelliferone, quercetin, scopoletinand myricetin were separated, whereas flavone glucoside immobilized. After dryness and determination Rf values of the above compounds, the plate was continued to be developped with toluene/ethyl acetate/formic acid (3:3:1), flavone glucoside and rhododendrin were located in the lower part of the plate. The Rf sequence of these compounds were: kaempferol-3-*o*-arabinoside, polystachoside > rhododendron > astrigalin > quercitrin > myricetrin > isoquercitrin > hyperside > rutin. To separate these components more completely, formic acid was added to solvent for development once again. Kaempferol-3-*o*-arabinoside and polystachoside were separated, while the Rf sequence of polystachoside and rhododendron was reversed. With polyamide thin layer and chloroform/methanol/butanone/ethyl acetate/formic acid (16:10:5:4:1) development, gosspetin-3-galactoside and catechin were also detected in *Rhododendron*. Analysis of 177 samples of the genus *Rhododendron* with HPTLC showed that the presence of monoglycosides of quercetin is a general character of flavonoids in the Chinese *rhododendrons*; some saxa are characterized by certain glycosides such as gossypetin and myricetin. Qualitative and semi-quantitative assessment of flavonoids is valuable in chemotaxonomy. The authors optimized solvent system, and ideal separations were achieved within three kinds of monoglycosides of quercetin, four flavonols and two coumarins. It was found that repeating development did not broaden sample spots, and the results were reproducible in the different plates. Moreover, about 20 samples might be applied in one plate (10 cm × 10 cm), the benefits are low cost and an increase in the number of samples processed, allowing more violent samples identified. So, TLC method is a potential technique for the determination of large numbers of plant materials.

Hi et al. [55] also have employed HPTLC scanning method to determine the contents of six flavnoids in 166 *Rhododendron* species. The methanol extract of each sample was spotted on a HPTLC silica gel plate alongside with the standard substances. Using the upper layer of petroleum ether (60–80 °C)/ether/formic acid (62:31:7) as the developing solvent A, farrerol, kaempferol and quercetin were well separated. The spots were determined by a single wavelength (366 nm) TLC scanner. The same plate was then further developed by developing solvent B: 7 ml of lower layer of chloroform/methanol/water (7:3:1) plus 0.5 ml of formic acid to separate polystachoside, quercitrin and hyperoside. Quantitative analysis of each spots was carried out with the same scanner by densitometric determination. The linear range was 0–1.75, 0.5–1.5, 0.5–2.5, and 1.25–7.5 μg for farrerol, kaempferol, quercetin, and polystachoside, respectively. The recovery of these four compounds ranged from 101.3 to 106.0%, and relative standard deviation was between 1.08 and 1.63%. The results revealed that *R. dauricum* L., collected in Helongjiang province, contains farrerol (0.02%), quercetin (0.02%), quercitrin (0.42%) and hyperoside (0.42%). With this method, kaempferol and polystachoside could not be detected in *R. dauricum* L.

3.3. High performance liquid chromatography (HPLC)

HPLC is regarded as a preferred technique due to its sensitivity, precision and specificity. HPLC methods with various strategies such as difference type of stationary phase, various composition of mobile phase, and a wide range of selective detector, make it suitable for analysis of active components

in crude herbs [56]. However, plant materials are complicated mixtures, composed of proteins, lipids, amino acids, carbohydrates, and, etc. Beyond the deleterious effects that these compounds may cause on the pump, the injector and the column, their presence will frequently interfere with the separation of the pharmaceutically active compounds. Consequently, selection of the sample preparation is always required before injection onto the HPLC system. The choice of pretreatment strategies and sample clean-up procedures will affect the efficiency and the selectivity of the chromatographic technique.

Li et al. [57] have established RP-HPLC method to determine farrerol in leaves of *R. dauricum* L. A 2.5 g of dried leaf sample were extractive distillated with 50 ml ether for 1 h. After filtration, the residues were extractive distillated twice. Then residues were washed with 20 ml ether twice. All filtrate solutions were mixed, and evaporated to remove the ether. Finally, the residues were dissolved in methanol, and diluted to 25 ml in volume. Twenty microliters of the sample solution was injected to HPLC system, in which Shim-Pach VP-ODS column, methanol/water (65.5:34.5) as mobile phase were used, and farrerol could be separated from coexistent constituents, with detection wavelength of 296 nm. The linear range of the method was 0.1–2.0 pg, average recovery was 96.60%, and R.S.D. ($n = 5$) was 1.07%. This method has been applied for the analysis of farrerol in leaves of *R. dauricum* L. and its preparations (Qing-bao capsules). The contents of farrerol in the two samples were 0.0547 mg/g and 0.0014 mg per capsule, respectively.

Quercetin in leaves of *R. dauricum* L. was also determined by HPLC [58]. Pretreatment was carried out by ultrasonic extraction with 40% ethanol for 30 min. With Altech C_{18} column (4.6 mm × 200 mm, 5 μm) and mobile phase of methanol/water/acetic acid (50:50:25), quercetin was separated, and detected at 370 nm. The average recovery was about 99.2% (R.S.D. = 0.98%, $n = 5$). The contents of quercetin in six samples collected in different production regions were investigated, which ranged from 0.0985 to 0.159%.

Pan [59] has studied RP-HPLC for the analysis of farrerol and quercetin in leaves of *R. dauricum* L. The methanol extraction was achieved by heating and reflux in the extractor for about 8 h, until the extract solution became colorless. After filtration and preconcentration, the sample solution was injected to HPLC system. The Nova-Pak C_{18} (4.6 mm × 250 mm, 2 μm) column was used for the separation of farrerol and quercetin; two different mobile phases, acetonitile/water (45:55) for farrerol and methanol/0.06% phosphoric acid (50:50) for quercetin were employed in this experiment. Furthermore, farreol was detected at 296 nm, and the detection wavelength for quercetin was 360 nm. So, farreol and quercetin could not be analyzed simultaneously in one run. Li et al. [60] have proposed RP-HPLC method to simultaneously determine the two flavonoids: quercetin and kaempferol in *Rhododendron*. The dried leaves were soaked in 75% ethanol for 72 h, and then the lixivium was filtrated

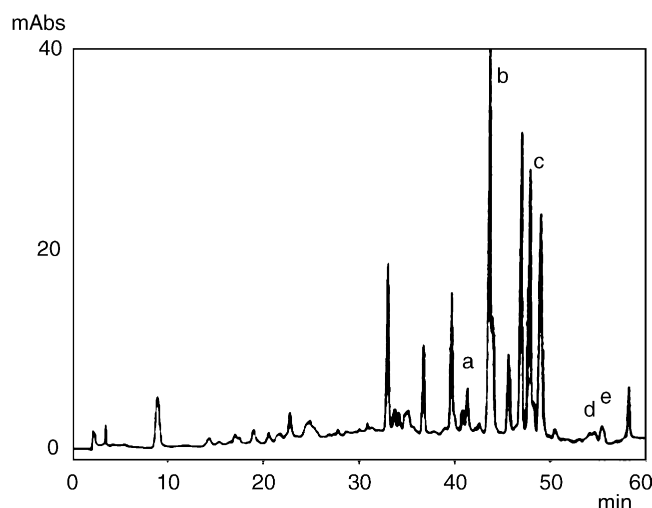


Fig. 8. HPLC chromatogram of flavonoid extract from leaves of rhododendron cultivar 'ScarletWonder' with low frost resistance (figure from Swiderski et al. [64]). Retention times of 40–50 and 50–60 min correspond to flavonoid glycosides and flavonoids, respectively: (a) naringin; (b) gossypin and rutin; (c) quercitrin; (d) naringenin; (e) quercetin.

and diluted. Filtrated through 0.45 μm filter, the sample solution was directly injected to HPLC system. The column was 4.6 mm × 250 mm, packed with Zorbax SB-C_{18} (5 μm). The mobile phase was a mixture of methanol/water (60:40). The flow rate was 0.7 ml/min. UV detector was performed at 360 nm. External standard was used and the calibration curve showed good linearity over range of 6.0–100 mg/L with detection limit in the range of 3.3–6.2 mg/L. The recoveries were 96.0% (R.S.D. = 2.1%) and 97.3% (R.S.D. = 4.5%) for quercetin and kaempferol, respectively. This method is simple, fast, sensitive and accurate, providing a scientific basis for industrial production and quality control for Jinjuan Liquor preparations for clinical uses. Moreover, Yang et al. [61], Pan [62], Lu and Zeng [63] and Peng et al. [64] also reported the assay results of farrerol, quercetin, kaempferol or rutin in *Rhododendron* species with RP-HPLC or HPLC approaches.

Swiderski et al. [65] studied the relationship between flavonoid concentration in *rhododendron* (*Rhododendron* L.) leaves and their frost resistance. The flavonoid content in various taxons of rhododendrons bred in Poland was compared with the extent of injury caused by the action of sub-zero temperatures. HPLC was used to analyze flavonoids and their glycosides, and the chromatogram was shown in Fig. 8. The results suggest that flavonoid concentrations in rhododendron tissues can be a measure of their resistance to low temperatures and can be used as a criterion in breeding.

3.4. Capillary electrophoresis (CE)

Although HPLC has been used for the determination of flavonoids in *Rhododendron*, use of HPLC for analysis of traditional Chinese medicines often has drawbacks such as

complicated pretreatment, solvent consumption, limited resolution and especially short column lifetime owing to easy contamination of numerous coexistent substances. CE is increasingly recognized as an important analytical separation technique because of its speed, efficiency, reproducibility, ultra-small sample volume, low consumption of solvent, and ease of removal of contaminants. These advantages indicate that CE is an ideal technique for the determination of complicated mixtures.

Our research group [66] have applied CE for the determination of some flavonoids and phenolic acids in *R. dauricum* L. As these active components could be easily oxidized electrochemically, electrochemical detection (ED) was employed. Compared with UV detection, for electroactive species, ED affords high sensitivity and good selectivity. The merit of good selectivity is especially noticeable for the analysis of plant materials, because large numbers of compounds such as proteins, carbohydrates, lipids, and, etc. in the plants are not electroactive, and cannot appear in the electropherogram, which makes the separations simpler and easier. With ultrasonic extraction and filtration, the sample solution was directly injected to the capillary. Farrerol, quercetin, syringic acid, vanillic acid, 4-hydroxybenzoic acid and protocatechuic acid were baseline separated within 16 min in a borax running buffer (pH 8.7). Operated in a wall-jet configuration, a 300 μm diameter carbon-disk electrode was used as the working electrode, which exhibited a good response at +950 mV (versus saturated calomel electrodes) for six analytes. Notably, excellent linearity was obtained over two orders of magnitude with detection limits (S/N = 3) ranged from 9×10^{-7} to 3.0×10^{-6} M for all analytes. The recoveries of the six components were ranged of 97.7–104.5% (R.S.D. in range of 1.9–4.6%). This method was successfully used in the analysis of *R. dauricum* L. with relatively simple extraction procedures, and the assay results were satisfactory as shown in Fig. 9.

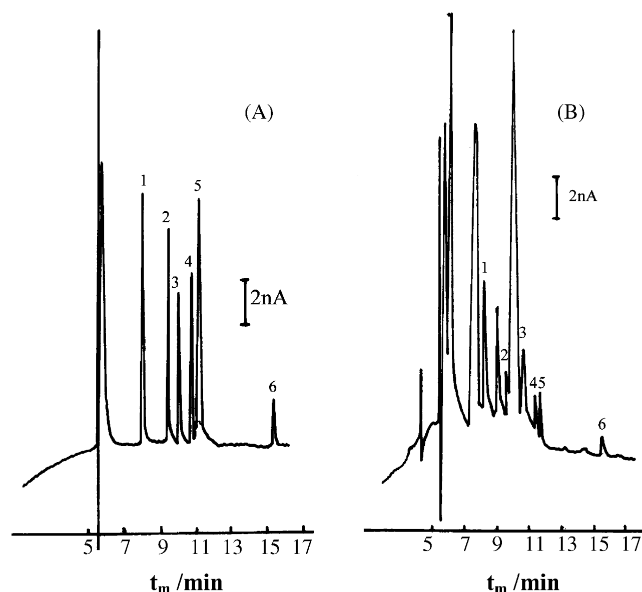


Fig. 9. The electropherograms of standard solution (A) and *R. dauricum* L. herb (B) (figure from Cao et al. [65]). Fused-silica capillary: 25 μm i.d. × 50 cm; working electrode: 300 μm diameter carbon disk electrode; working electrode potential is +0.95 V (vs. SCE); running buffer: 50 mmol L^{-1} (pH 8.7); separation voltage: 14 kV; injection time: 14 kV/6 s; concentrations of six analytes: 1.0×10^{-4} mol L^{-1} each. Peak identification: 1, farrerol; 2, syringic acid; 3, vanillic acid; 4, 4-hydroxy benzoic acid; 5, quercetin; 6, protocatechuic acid.

The botanical origin of honey is one of its main quality parameters, and its price is very often related to this floral origin [67]. P. Andrade et al. [68] have analyzed 26 phenolic compounds from honey sample with 11 floral origins by CZE. To extract the phenolic compounds from the honey samples, liquid–solid phase extraction was applied. The available honey samples were thoroughly mixed with five parts of water, until completely fluid, and filtered through cotton to remove solid particles. The filtrate was then passed through a pre-column (25 cm × 2 cm) of Amberlite XAD-2. The phenolic compounds present in honey remained in the column while sugars and other polar compounds were eluted with aqueous solvent. The phenolic fraction was eluted with methanol, concentrated under reduced pressure, and purified by dissolving in methanol and passing through another column (15 cm × 1 cm) of Sephadex LH-20. The phenolic fraction was then evaporated to dryness under reduced pressue, redissolved in methanol and analyzed by CZE. All the phenolics were separated on a fused-silica capillary (50 cm × 50 μm) using 100 mM borate buffer (pH 9.5) plus 20% methanol within 20 min. The phenolic compounds from heather, lavender, acacia, rape, sunflower, rosemary, citrus, *rhododendron*, thyme, chestnut-tree and calluna honey samples were separated with CE method, and the electropherogram obtained from *Rhododendron* honey sample was shown in Fig. 10. The phenolic compounds presented in the honey were identified by their

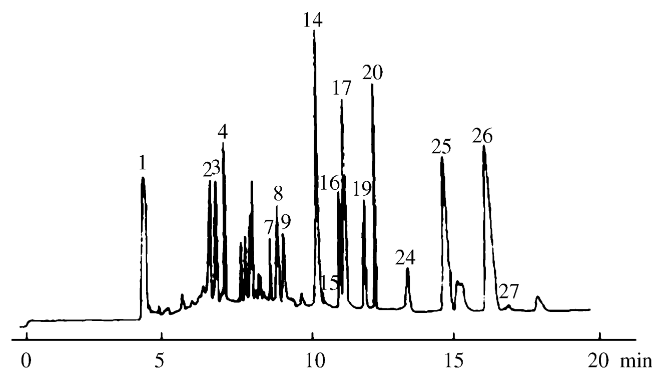


Fig. 10. The electropherogram of phenolic compounds in *Rhododendron* L. (figure from Andrade et al. [67]). Fused-silica capillary: 50 μm i.d. × 50 cm; running buffer: 100 mmol L^{-1} borax buffer (pH 9.5) plus 20% methanol; separation voltage: 20 kV; detection at 280 nm. Peak identifications: 1, hydroxymethylfurfural; 2, phenylethylcaffeate; 3, dimethylallylcaffeate; 4, pinobanksin; 5, naringenin; 6, hesperetin; 7, cinnamic acid; 8, chlorogenic acid; 9, *m*-coumaric acid; 10, quercetin; 11, luteolin; 12, syringic acid; 13, ferulic acid; 14, pinocembrin; 15, *o*-coumaric acid; 16, kaempferol; 17, *p*-coumaric acid; 18, apigenin; 19, vanillic acid; 20, chrysin; 21, galangin; 22, ellagic acid; 23, rosmarinic acid; 24, *p*-hydroxybenzoic acid; 25, caffeic acid; 26, gallic acid; 27, 2,4-dihydroxybenzoic acid.

UV spectra recorded with the diode array detector and by electrophoretic comparisons (migration times) with authentic markers, however, quantitative analysis of these components were not carried out. The authors paid attention to establish correlations between the phenolics profiles and the botanical origin of the honey. From their electropherograms, the differences in phenolics profiles are very clear. On one hand, whether phenolic acids or flavonoids have higher contents in honey samples indeed indicated the floral characters; On the other hand, some individual honey showed potential floral markers. It is proposed that thyme honey was characterized by the presence of rosmarinic acid, heather honey by ellagic acid, citrus honey by hesperetin, and lavender honey by naringenin, respectively. The studies have revealed that the analysis of phenolic compound constituents is a very promising technique for studying the geographical and floral origin of honey.

4. Conclusion

More than 90 chemical constituents in *R. dauricum* L. have been detected and identified, and many pharmacologists have been studying the biological activity of these compounds. On the other hand, research on quantitative methods of these active compounds in *R. dauricum* L. is continued. So far, only about ten papers concerning the quantitative determination of active compounds in *R. dauricum* L. have been found. Moreover, most of analytical methods only concentrate on determination of a total content of the flavonoids, volatile oils and a fraction of flavonoids and phenolic acids. Quantitative method for the majority of important pharmaceutically active components in *R. dauricum* L. has not been established yet. It is an essential but challenging task for the analytical researchers to piece together a complete chemical profile of active components in *R. dauricum* L.

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