

超高效液相色谱-四极杆-飞行时间质谱法 分析葫芦巴成分

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摘要:采用超高效液相色谱-四极杆-飞行时间质谱法(UPLC-Q-TOF MS)对葫芦巴的醇提组分和除糖后的水提组分进行定性分析,并以甘草苷为内标,对鉴定出的成分进行半定量分析。采用 Unitary C18 色谱柱(4.6 mm×150 mm×5 μm),以乙腈-0.1%甲酸/水为流动相进行线性梯度洗脱,流速 0.5 mL/min,电喷雾离子源(ESI)负离子模式检测。定性分析结果表明,葫芦巴的醇提组分和水提组分的总离子流图相似,共鉴定出 36 种化合物,包括 12 种黄酮类成分和 24 种皂苷类成分。实验选择了几种代表性的黄酮类和皂苷类化合物,对其结构进行详细阐述,并推断其可能的质谱裂解规律。半定量分析结果表明,两种提取物中主成分的相对含量不同,黄酮类化合物在乙醇提取物中的相对含量之和是水提物中的 1.13 倍,皂苷类化合物在乙醇提取物中的相对含量之和是水提物中的 1.32 倍。该方法简便、快捷、灵敏,可为葫芦巴的药效物质基础研究和开发应用提供理论依据。

关键词:葫芦巴;黄酮;皂苷;超高效液相色谱-四极杆-飞行时间质谱(UPLC-Q-TOF MS)

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Chemical Constituents of Fenugreek Using UPLC-Q-TOF MS

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Abstract: Fenugreek in invigorating kidney, falling blood sugar, etc, have very good medicinal value. Much attention has been paid to saponins, flavonoids, the separation and purification, the compatibility mechanism of these components have been studied in depth, but the preparation is complicated, time-consuming, labor-intensive and poor economic. In this research, compounds in the ethanol extract and the water extract after sugar removal of fenugreek were qualitatively analyzed by ultra performance liquid chro-

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matography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOFMS). And the liquiritin was selected as the internal standard for semi-quantitative analysis of all the identified components. Chromatographic separation was performed on a Unitary C18 column (4.6 mm×150 mm×5 μm). Acetonitrile and 0.1% (volume fraction) formic acid solution were used as the mobile phase for gradient elution with the flow rate of 0.5 mL/min. The MS analysis was carried out by the electrospray ionization (ESI) source in negative mode. The qualitative analysis results showed that the total ion chromatograms of the ethanol extract and the water extract after sugar removal of fenugreek were similar with each other, and 36 compounds were identified, including 12 flavonoids and 24 saponin components. Taking typical flavonoids and saponins as examples, the structures of flavonoids and saponin were described in detail, and the possible breaking rules of flavonoids and saponin were deduced. The results of semi-quantitative analysis showed that the relative content of the main components in the two extracts was different. The sum of the relative contents of the flavonoids in the ethanol extract was 1.13 times that in the aqueous extract. The sum of the relative contents of the saponin compounds was 1.32 times that in the aqueous extract. The method is very simple, rapid and sensitive, and the obtained results will provide a theory basis for further research on fenugreek, as well as development and application research of fenugreek.

Key words: fenugreek; flavonoid; saponins; ultra performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF MS)

葫芦巴 (*Trigonella foenum-graecum*, L.), 别名苦豆、香豆子、香草, 为豆科蝶形花亚科一年生草本植物。葫芦巴种子是我国传统中药, 具有抗肿瘤、降血糖、抗氧化等药用价值, 已被多版《中国药典》收录。此外, 葫芦巴还被广泛用于食品工业^[1-2]。现代研究表明, 葫芦巴的主要成分为糖类、蛋白质类、脂类、皂苷类、黄酮类、生物碱类和挥发性有机成分等, 其中, 含量最高的 3 类药效活性组分依次是多糖、皂苷和黄酮。目前, 对葫芦巴中葫芦巴碱、多糖、挥发油等成分的研究较多^[3-5], 而对黄酮和皂苷类化合物的报道较少。

研究中药化学成分的传统方法是用溶剂分配和柱色谱法将其逐一分离为单一化合物, 然后采用化学方法对其结构进行鉴定, 操作繁琐、分离效率和自动化程度低, 且对微量组分的分离困难。高效液相色谱-质谱法具有高效、快速、灵敏等优点, 可用于中药天然产物有效成分的定性、定量分析^[6-7]。

有文献报道^[8-10], 葫芦巴多糖的提取多采用水提取工艺, 而黄酮和皂苷多采用高浓度的乙醇提取工艺。本实验室在前期研究中发

现, 与单组分比较, 葫芦巴多糖、皂苷、黄酮 3 个组分分配伍对糖尿病的治疗效果更好^[11]。为此, 本工作利用水提和醇提两种方法提取葫芦巴中的化学成分, 采用超高效液相色谱-四极杆飞行时间质谱 (UPLC-Q-TOF MS) 技术分析提取物中的黄酮和皂苷类化合物, 以期找到一种简便快速的提取工艺能将 3 种组分同时提取出来, 为深入开展葫芦巴活性成分的提取和药效学研究奠定基础, 同时为研究葫芦巴的药效物质基础和开发应用提供理论依据。

1 实验部分

1.1 仪器与装置

Acquity UPLC 液相色谱仪、SYNAPT G2-S HDMS 质谱仪; 均为美国 Waters 公司产品; Unitary C18 分析柱: 华普新科技有限公司产品。

1.2 材料与试剂

葫芦巴种子: 购自长春市医药药材供销公司, 经吉林大学药学院王广树教授鉴定为葫芦巴种子; 甲醇和乙酸: 均为色谱纯, 美国 Fisher

公司产品;超纯水:由 Milli-Q 系统制备;其他试剂均为色谱级。

1.3 实验方法

1.3.1 溶液制备 葫芦巴醇提组分的制备:精密称取 5 g 葫芦巴粗粉,用 10 倍量的 70%乙醇回流提取 3 次,每次 2 h,减压抽滤,合并滤液、浓缩、冷冻干燥,得到葫芦巴醇提组分。

葫芦巴水提组分的制备:精密称取 5 g 葫芦巴粗粉,用 50 倍量的水在 10 °C 冷浸提取 3 次,每次 1 h,以 4 000 r/min 离心 10 min,收集上清液、浓缩。向溶液中加入 95%乙醇至乙醇浓度为 70%,边加边搅拌,静置过夜,以 4 000 r/min 离心 10 min,收集上清液、浓缩、冷冻干燥,得到葫芦巴除糖后的水提组分。

甘草苷内标溶液的制备:精密称取 1 mg 甘草苷标准品,用 50%甲醇溶解并稀释至 25 mg/L。

供试品溶液的制备:精密称取适量的葫芦巴醇提组分和除糖后的水提组分,用 50%甲醇溶解,加入适量的甘草苷内标溶液,使醇提组分和水提组分供试品溶液的最终生药浓度为 10 g/L,甘草苷内标物的最终浓度为 5 mg/L,过 0.22 μm 滤膜,待测。

1.3.2 UPLC-MS 和 UPLC-MS/MS 分析 色谱条件:Unitary C18 分析柱(4.6 mm×150 mm×5 μm);流动相:A 为 0.1%甲酸,B 为乙腈;洗脱程序:0~5 min(10%~15%B),5~10 min(15%B),10~20 min(15%~20%B),20~25 min(20%~23%B),25~40 min(23%~24%B),40~48 min(24%~25%B),48~64 min(25%~40%B),64~65 min(40%~55%B),65~70 min(55%~60%B),70~75 min(70%B),75~80 min(70%~80%B),80~85 min(80%~100%B);流速 0.5 mL/min;柱温 25 °C;进样量 5 μL。

质谱条件:电喷雾离子源(ESI);四极杆飞行时间串联质量分析器;负离子模式检测;离子源温度 120 °C;脱溶剂气温度 250 °C;锥孔气(N₂)流速 50 L/h;脱溶剂气(N₂)流速为 600 L/h;毛细管电压 2.0 kV;锥孔电压 60 V。

2 结果与讨论

2.1 葫芦巴醇提、水提组分化学成分的分析

采用 UPLC-Q-TOF 负离子模式分析加入

甘草苷后的葫芦巴醇提组分和水提组分供试品溶液,得到的总离子流图示于图 1。可见,内标化合物甘草苷的保留时间为 24.25 min,位于化合物 11 和 12 之间。各化合物得到了较好的分离,两种供试品溶液的总离子流图相似,区别是保留时间 75 min 后,醇提组分的色谱峰比水提组分多,说明醇提组分中极性小的化合物比水提组分多。本研究共鉴定出 36 种化合物,包括 12 种黄酮类化合物和 24 种皂苷类化合物,具体信息列于表 1。

2.2 黄酮类化合物结构鉴定

葫芦巴黄酮的主要苷元为芹菜素(apigenin, api)和木犀草素(luteolin, lut)。以芹菜素为苷元的黄酮,其特征碎片为 m/z 383 [M-H-CHOH-CHO-CH₂OH]⁻ (M-H-120-90)、 m/z 353 [M-H-CHOH-CHOH-CHO-CH₂OH]⁻ (M-H-120-120);以木犀草素为苷元的黄酮,其特征碎片为 m/z 357 [M-H-CHOH-CHO-CH₂OH]⁻ (M-H-90)、 m/z 327 [M-H-CHOH-CHOH-CHO-CH₂OH]⁻ (M-H-120)。葫芦巴黄酮糖苷主要连接在 6 位和 8 位上,易发生糖环内部裂解,糖环内部裂解碎片丢失具有一定的规律性,组成糖链的单糖分为戊糖(木糖、阿拉伯糖)和己糖(葡萄糖、半乳糖、鼠李糖),戊糖出现 60、90 u 碎片丢失;己糖出现 60、90、120 u 碎片丢失^[12-13],其中鼠李糖出现 74、104 u 碎片丢失,结合准分子离子峰,有助于快速发现和鉴定碳苷类化合物。此外,结合相关文献^[14-15],可根据碎片离子的相对丰度强弱,推测黄酮类化合物中糖苷的位置:如准分子离子 m/z 563 的碎片离子峰 m/z 473 相对丰度明显强于碎片离子峰 m/z 443 时,可推测戊糖连接在 6 位 C 上;准分子离子 m/z 447 的碎片离子峰 m/z 327 相对丰度明显强于碎片离子峰 m/z 357 时,可推测己糖连接在 8 位 C 上。

葫芦巴黄酮准分子离子峰主要包括 m/z 593、563、557、461、431、447,其中含有大量的同分异构体,由于缺少对照品,仅鉴定了苷元的类型、糖的类型及糖苷位置,尚不能确定其具体结构。通过分析黄酮类化合物在 MS² 模式下的碎片离子,并查阅相关文献^[15-18],本实验最

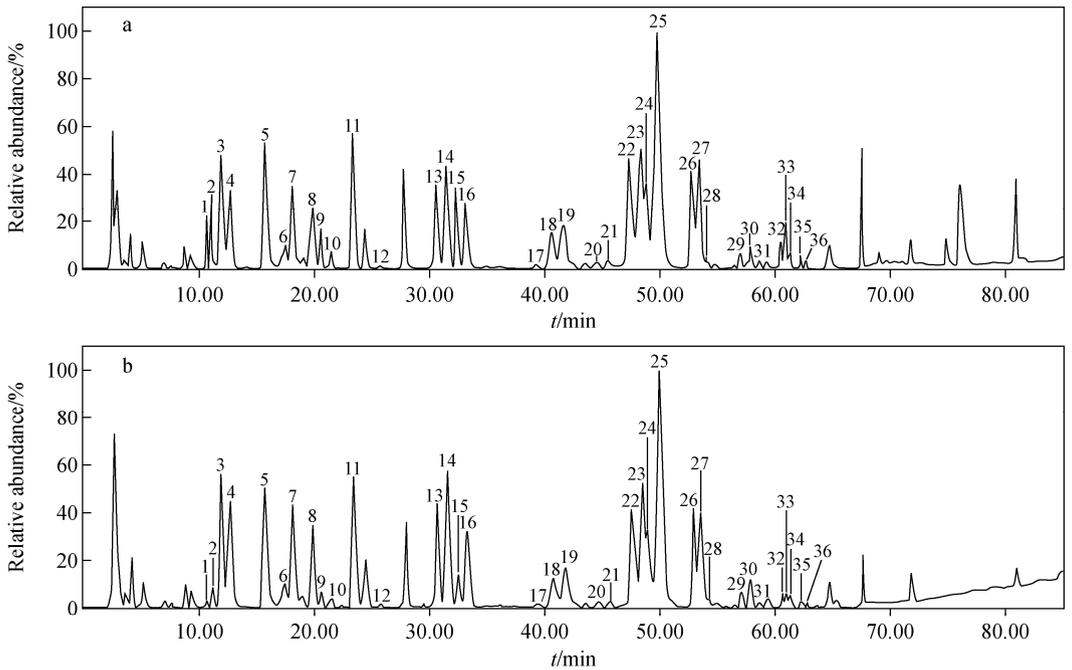


图 1 葫芦巴加入甘草苷后,醇提组分(a)和水提组分(b)的总离子流图
Fig. 1 Chromatograms of ethanol extract (a) and water extract (b)
after sugar removal with liquiritin of fenugreek

终鉴定出 11 种黄酮类化合物的结构,示于图 2。第 12 个推测为类黄酮类化合物(槲皮素类化合物),因其双键位置不确定,未列于图 2 中。

准分子离子为 m/z 593、563、557、431 的葫芦巴黄酮类成分均出现了 m/z 383、353 碎片离子,由此判断它们的苷元为芹菜素。以化合物 3 为例,其碎片离子为 m/z 593、503、473、383、353,出现了两次 90、120 u 中性丢失,即发生两次糖环内部裂解,表明其含有 2 个己糖,分别连接在 6 位和 8 位 C 上,其质谱图示于图 3。结合参考文献^[12],最终确定化合物 3 为芹菜素-6,8-C-二葡萄糖苷。

准分子离子峰 m/z 563 出现了 m/z 503、473、443、383、353 碎片离子,中性丢失依次为 60、90、120 u,推测其含有 1 个戊糖和 1 个己糖结构;准分子离子峰 m/z 577 出现了 m/z 503 碎片离子,中性丢失为 74 u,推测其含有鼠李糖结构;准分子离子峰 m/z 431 先后发生中性丢失 90、120 u,推测其含有 1 个己糖结构。

化合物 7、8 均出现准分子离子峰 m/z 447,碎片离子为 m/z 357[M-H-CHOH-CHO-CH₂OH]⁻(M-H-90)、 m/z 327[M-

H-CHOH-CHOH-CHO-CH₂OH]⁻(M-H-120),确定化合物 7 和 8 的苷元为芹菜素。以化合物 8 为例,其 MS/MS 碎片离子 m/z 327 的相对丰度明显强于 m/z 357,推测己糖连接在 8 位 C 上,最终鉴定该化合物为木犀草素-8-C-己糖苷,其中己糖苷为葡萄糖或半乳糖,示于图 4。

2.3 皂苷类化合物结构鉴定

葫芦巴皂苷为甾体皂苷,包括呋喃皂苷、螺甾皂苷,苷元种类丰富。通常以一对非对映异构体(C-25S 和 C-25R)的形式存在,在 ODS 柱上 S 构型的皂苷较 R 构型出峰早,由此可判断一对非对映异构体的构型^[19]。葡萄糖、鼠李糖、木糖构成了糖基,其连接在 3 位和 26 位羟基上,裂解时糖环依次整个脱落,由中性丢失顺序可推断糖基连接顺序,同时,通常将最后 1 个丢失的糖基归于 26 位羟基。

通过分析 UPLC-Q-TOF MS/MS 模式下的碎片离子和保留时间,并结合参考文献^[19-21],推断皂苷中的糖基数量、单糖类型、连接位点、C-25S/R 构型以及苷元类型等,最终鉴定出 24 种皂苷类化合物,结构示于图 5。

表 1 葫芦巴加入甘草昔后,醇提组分和水提组分的 UPLC-MS² 数据Table 1 UPLC-MS² data of ethanol extract and water extract after sugar removal with liquiritin of fenugreek

序号 No.	保留时间 <i>t_R</i> /min	准分子离子 Parent ions (<i>m/z</i>)		碎片离子 Daughter ions (<i>m/z</i>)	化合物名称 Compounds	醇提物中各化合物的相对峰面积 Relative peak area of ethanol extract/%		水提取物中各化合物的相对峰面积 Relative peak area of water extract/%	
1	10.71	593	503,473,383,353	503,473,383,353	Vicenin II or isomer; apigenin 6, 8-di C-hexoside	11.27	8.43		
2	11.20	593	503,473,383,353	503,473,383,353	Vicenin II or isomer; apigenin 6, 8-di C-hexoside	27.82	25.93		
3	11.87	593	503,473,383,353	503,473,383,353	Vicenin II or isomer; apigenin 6, 8-di C-hexoside	301.78	291.70		
4	12.67	593	503,473,383,353	503,473,383,353	Vicenin II or isomer; apigenin 6, 8-di C-hexoside	175.90	213.69		
5	15.59	563	503,473,443,383,353	503,473,443,383,353	Vicenin I or isomer; apigenin 6, 8-di C-hexosyl-pentosyl	491.95	399.84		
6	17.50	563	503,473,443,383,353	503,473,443,383,353	Vicenin I or isomer; apigenin 6, 8-di C-hexosyl-pentosyl	76.81	68.80		
7	18.14	447	369,357,327,298	369,357,327,298	Luteolin-8-C-hexoside	317.96	291.33		
8	19.94	447	369,357,327,298	369,357,327,298	Luteolin-8-C-hexoside	245.07	205.45		
9	20.53	577	559,503,383,353,473,457	559,503,383,353,473,457	Apigenin 6, 8-di C-rhamnopyranosyl-glucosyl	24.34	29.29		
10	21.39	563	503,473,443,383,353	503,473,443,383,353	Vicenin III or isomer; apigenin 8-C-pentoxide-6-C-hexoside	38.07	18.68		
11	23.34	431	413,341,311	413,341,311	Apigenin-6/8-C-glucoside	424.19	339.05		
12	25.90	461	341,371,327	341,371,327	6/8-C-Glycosylated flavonoid	6.21	7.82		
13	30.67	905	951,773,611,449	951,773,611,449	Trigoneoside Ia; (25S)-5 α -furost-2 α , 3 β , 22, 26-tetraol, 3-O-[β D-Xylopyranosyl-(1 \rightarrow 6)- β D-glucopyranoside]-26-O- β D-glucopyranoside	219.25	212.56		
14	31.54	905	951,773,611,449	951,773,611,449	Trigoneoside Ib; (25R)-5 α -furost-2 α , 3 β , 22, 26-tetraol, 3-O-[β D-Xylopyranosyl-(1 \rightarrow 6)- β D-glucopyranoside]-26-O- β D-glucopyranoside	299.14	298.36		
15	32.50	919	965,773,611,449	965,773,611,449	Trigoneoside Xa; (25S)-5 α -furost-2 α , 3 β , 22, 26-tetraol, 3-O-[α L-Rhamnopyranosyl-(1 \rightarrow 2)- β D-glucopyranoside]-26-O- β D-glucopyranoside	61.11	56.54		

续表 1

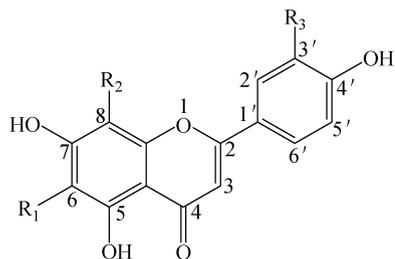
序号 No.	保留时间 t_R /min	准分子 离子 Parent ions (m/z)	碎片离子 Daughter ions (m/z)	化合物名称 Compounds	醇提取物中各化 合物的相对峰 面积 Relative peak area		水提取物中各化 合物的相对峰 面积 Relative peak area	
					of ethanol extract/%	of water extract/%	of ethanol extract/%	of water extract/%
16	33.24	919	965, 773, 611, 449	Trigoneoside Xb: (25R)-5 α -furost-2 α , 3 β , 22, 26-tetraol, 3-O-[α -L-Rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside]-26-O- β -D-glucopyranoside	183.67	203.75		
17	39.27	1517	1563, 1385, 1223, 1061, 899, 753, 429	Trigoneoside VI: 5, 25(27)-dien-furost-3 β , 22, 26-triol, 3-O-(β -D-Glucopyranosyl-(1 \rightarrow 6)-[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranosyl- (1 \rightarrow 3)- β -D-glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -glucopyranoside	18.24	11.35		
18	40.67	1519	1565, 1387, 1063, 901, 755, 593	Trigoneoside Va: (25S)-furost-5-en-3 β , 22, 26-triol, 3-O-(β -D-Glucopyranosyl-(1 \rightarrow 6)-[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranosyl- (1 \rightarrow 3)- β -D-Glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D- glucopyranoside)-26-O-D- β -glucopyranoside	180.94	115.82		
19	41.98	1519	1565, 1387, 1063, 901, 755, 593	Trigoneoside Vb: (25R)-furost-5-en-3 β , 22, 26-triol, 3-O-(β -D-Glucopyranosyl-(1 \rightarrow 6)-[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranosyl- (1 \rightarrow 3)- β -D-glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D- glucopyranoside)-26-O-D- β -glucopyranoside	241.52	164.47		
20	44.72	1225	1271, 1063, 901, 755, 593, 431	Trigoneoside XIII a: (25S)-furost-5-en-3 β , 22, 26-triol, 3-O-(β -D-glucopyranosyl (1 \rightarrow 3)- β -D-glucopyranosyl (1 \rightarrow 4)-[α -L-rhamnopyranosyl(1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -D-glucopyranoside	22.31	10.00		
21	45.66	1225	1271, 1063, 901, 755, 593, 431	Trigoneoside XIII b: (25R)-furost-5-en-3 β , 22, 26-triol, 3-O-(β -D-glucopyranosyl (1 \rightarrow 3)- β -D-glucopyranosyl (1 \rightarrow 4)-[α -L-rhamnopyranosyl(1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -D-glucopyranoside	24.82	13.91		

续表 1

序号 No.	保留时间 t_R /min	准分子离子		化合物名称 Compounds	醇提取物中各化合物的相对峰面积		水提取物中各化合物的相对峰面积	
		Parent ions (m/z)	Daughter ions (m/z)		peak area of ethanol extract/%	Relative area of water extract/%		
22	47.51	1063	1109, 901, 755, 593, 431	Trigoneoside W a; (25S)-furost-5-en-3 β , 22, 26-triol, 3- <i>O</i> -(β -D-Glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26- <i>O</i> - β -glucopyranoside	355.71	267.91		
23	48.48	1063	1109, 901, 755, 593, 431	Glycoside F; (25R)-furost-5-en-3 β , 22, 26-triol, 3- <i>O</i> -(β -D-Glucopyranosyl-(1 \rightarrow 6)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26- <i>O</i> - β -glucopyranoside	322.15	292.45		
24	48.95	1047	1093, 901, 755, 593, 431	(25S)-Furost-5-en-3 β , 22, 26-triol, 3- <i>O</i> -(α -L-Rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26- <i>O</i> - β -glucopyranoside	171.97	81.95		
25	49.91	1047	1093, 901, 755, 593, 431	(25R)-Furost-5-en-3 β , 22, 26-triol, 3- <i>O</i> -(α -L-Rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26- <i>O</i> - β -glucopyranoside	1211.23	878.04		
26	52.93	901	947, 755, 593, 431	Glycoside D; (25S)-furost-5-en-3 β , 22, 26-triol, 3- <i>O</i> -(α -L-Rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside)-26- <i>O</i> - β -D-glucopyranoside	336.57	242.38		
27	53.51	901	947, 755, 593, 431	Trigofenoside A; (25R)-furost-5-en-3 β , 22, 26-triol, 3- <i>O</i> -(α -L-Rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside)-26- <i>O</i> - β -D-glucopyranoside	395.03	257.60		
28	54.24	903	949, 757, 595, 433	(25S)-5 α -Furost-3 β , 22, 26-triol, 3- <i>O</i> -(α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside)-26- <i>O</i> - β -D-glucopyranoside or its isomer	20.24	17.11		
29	57.03	887	933, 755, 593, 431	26- <i>O</i> - β -D-Glucopyranosyl-25(27)-ene-5 α -furostane-3 β , 22 α , 26-triol, 3- <i>O</i> - β -D-Xylopyranosyl-(1 \rightarrow 6)- β -D-27-glucoopyranoside	46.25	32.66		

续表 1

序号 No.	保留时间 t_R /min	准分子 离子 Parent ions (m/z)	碎片离子 Daughter ions (m/z)	化合物名称 Compounds	醇提取物中各化 合物的相对峰 面积 Relative peak area		水提取物中各化 合物的相对峰 面积 Relative peak area	
					of ethanol extract/%	of water extract/%	of ethanol extract/%	of water extract/%
30	57.86	901	947, 755, 593, 449	25(S)-Furost-4-en-3 β , 22, 26-triol, 3-O-[α -L-Rhamnopyranosyl(1 \rightarrow 2)- β -D-glucopyranoside]- 26-O- β -D-glucopyranosideor its isomer	54.83	54.83	54.49	54.49
31	58.56	1501	1547, 1369, 1045, 883, 575	5, 25(27)-Dien-furost-3 β , 22, 26-triol, 3-O-(β -D-Rhamnopyranosyl-(1 \rightarrow 6)-[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranosyl- (1 \rightarrow 3)- β -D-glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -glucopyranoside	18.93	18.93	8.85	8.85
32	60.68	1045	1091, 883, 737, 555	(25S)-Furost-5, 20(22)-dien-3 β , 26-diol, 3-O-(β -D-Glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -glucopyranoside	35.47	35.47	15.39	15.39
33	61.05	1029	1075, 883, 737, 575	(25R)-Furost-5, 20(22)-dien-3 β , 26-diol, 3-O-(α -L-Rhamnopyranosyl-(1 \rightarrow 6)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -glucopyranoside	71.52	71.52	31.45	31.45
34	61.35	1029	1075, 883, 737, 575	Pseudoprotodioscin: (25R)-furost-5, 20(22)-dien-3 β , 26-diol, 3-O-(α -L-Rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl- (1 \rightarrow 2)]- β -D-glucopyranoside)-26-O- β -D-glucopyranoside	22.92	22.92	10.01	10.01
35	62.29	883	929, 737	(25S)-Furost-5, 20(22)-dien-3 β , 26-diol, 3-O-[α -L-Rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside]-26-O- β -D-glucopyranoside	22.41	22.41	10.48	10.48
36	62.78	883	929, 737	pseudoprotoprogenin III: (25R)-furost-5, 20(22)-dien-3 β , 26-diol, 3-O-[α -L-Rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside]-26-O- β -D-glucopyranoside	14.71	14.71	6.06	6.06



1. R₁=hex; R₂=hex; R₃=H
2. R₁=hex; R₂=hex; R₃=H
3. R₁=hex; R₂=hex; R₃=H
4. R₁=hex; R₂=hex; R₃=H
5. R₁=pen or hex; R₂=pen or hex; R₃=H
6. R₁=pen or hex; R₂=pen or hex; R₃=H
7. R₁=H; R₂=hex; R₃=OH
8. R₁=H; R₂=hex; R₃=OH
9. R₁=rha or glc; R₂=rha or glc; R₃=H
10. R₁=hex; R₂=pen; R₃=H
11. R₁=glc or H; R₂=glc or H; R₃=H

图2 葫芦巴中黄酮类成分的化学结构

Fig. 2 Chemical structures of flavonoids in fenugreek

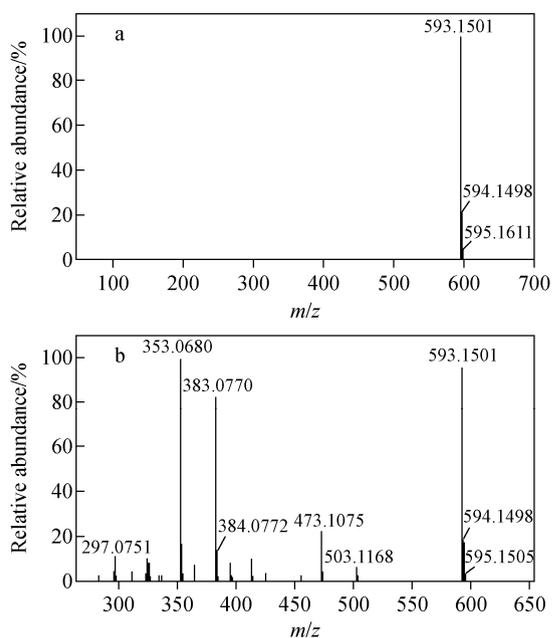


图3 化合物3的MS(a)和MS/MS(b)质谱图

Fig. 3 MS (a) and MS/MS (b) spectra of compound 3

以化合物13为例,其MS/MS谱图示于图6。在MS模式下,出现准分子离子峰 m/z 905及碎片离子峰 m/z 951 $[M+HCO_2H]^-$, MS²模式下出现碎片离子 m/z 773 $[M-132]^-$ 、 m/z 611 $[M-132-162]^-$ 、 m/z 449 $[M-132-162-162]^-$,推测其先后失去1分子木

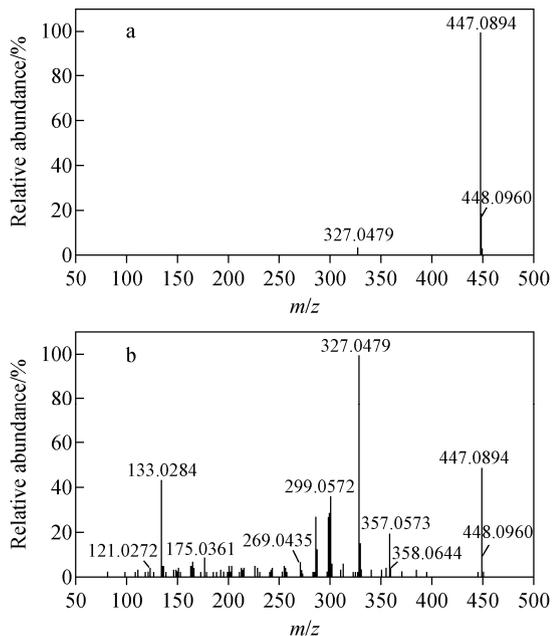


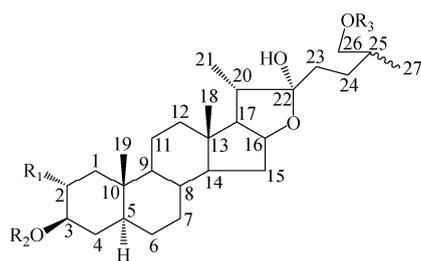
图4 化合物8的MS(a)和MS/MS(b)谱图

Fig. 4 MS (a) and MS/MS (b) spectra of compound 8

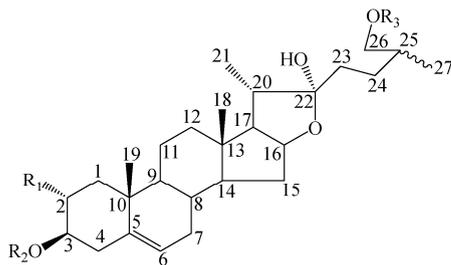
糖、1分子葡萄糖、1分子葡萄糖,最后1个葡萄糖通常连接在C-26羟基上。此外,根据R/S构型异构体在ODS柱上的出峰特点,即25S构型的化合物出峰早于25R构型的化合物,最终确定该皂苷为葫芦巴皂苷Ia:25(S)-5 α -呋甾-2 α , 3 β , 22, 26-四醇, 3-O- $[\beta$ -D-木吡喃糖基-(1 \rightarrow 6)- β -D-吡喃葡萄糖苷]-26-O- β -D-吡喃葡萄糖苷。

2.4 乙醇提取物和水提物主要成分的相对含量分析

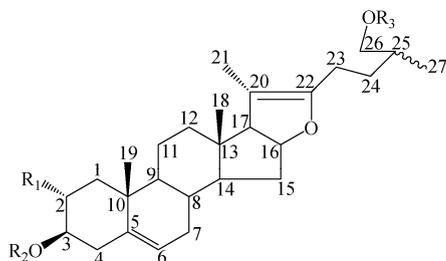
分别向乙醇提取物和水提物的供试品溶液中加入甘草苷标准品溶液,使两种提取物溶液的最终生药浓度为10.0 g/L,甘草苷的最终浓度为5.0 mg/L。以甘草苷的峰面积为标准,将两种提取物溶液中主要化合物的峰面积与甘草苷峰面积对比,得到各化合物的相对峰面积值,列于表1。从表1可以看出,与水提物相比,乙醇提取物中化合物5、10、11的相对含量明显较高,其他黄酮类化合物的相对含量没有明显变化;乙醇提取物中黄酮类化合物的相对含量之和是水提物中的1.13倍,乙醇提取物中皂苷类化合物相对含量之和是水提物中的1.32倍。



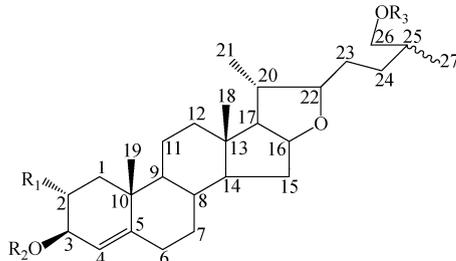
13. 25*S* R₁=OH; R₃=Glc; R₂=Xly(1→6)Glc
 14. 25*R* R₁=OH; R₃=Glc; R₂=Xly(1→6)Glc
 15. 25*S* R₁=OH; R₃=Glc; R₂=Xly(1→2)Glc
 16. 25*R* R₁=OH; R₃=Glc; R₂=Xly(1→2)Glc
 28. 25*R* or 25*S* R₁=H; R₃=Glc; R₂=Rha(1→2)Glc
 29. 25(27)-en R₁=H; R₃=Glc; R₂=Xly(1→6)Glc



17. 25(27)-en R₁=H; R₃=Glc; R₂=Glc(1→6)
 [Xyl(1→4)]Glc(1→4)[Rha(1→2)]Glc
 18. 25*S* R₁=H; R₃=Glc; R₂=Glc(1→6)[Xyl(1→4)]
 Glc(1→4)[Rha(1→2)]Glc
 19. 25*R* R₁=H; R₃=Glc; R₂=Glc(1→6)[Xyl(1→4)]
 Glc(1→4)[Rha(1→2)]Glc
 20. 25*S* R₁=H; R₃=Glc; R₂=Glc(1→3)Glc(1→4)[Rha(1→2)]Glc
 21. 25*R* R₁=H; R₃=Glc; R₂=Glc(1→3)Glc(1→4)[Rha(1→2)]Glc
 22. 25*S* R₁=H; R₃=Glc; R₂=Glc(1→4)[Rha(1→2)]Glc
 23. 25*R* R₁=H; R₃=Glc; R₂=Glc(1→6)[Rha(1→2)]Glc
 24. 25*S* R₁=H; R₃=Glc; R₂=Rha(1→4)[Rha(1→2)]Glc
 25. 25*R* R₁=H; R₃=Glc; R₂=Rha(1→4)[Rha(1→2)]Glc
 26. 25*S* R₁=H; R₃=Glc; R₂=Rha(1→2)Glc
 27. 25*R* R₁=H; R₃=Glc; R₂=Rha(1→2)Glc
 31. 25(27)-en R₁=H; R₃=Glc; R₂=Rha(1→6)[Xyl(1→4)]
 Glc(1→3)Glc(1→4)[Rha(1→2)]Glc
 32. 25*S* R₁=H; R₃=Glc; R₂=Glc(1→4)[Rha(1→2)]Glc
 33. 25*R* R₁=H; R₃=Glc; R₂=Glc(1→6)[Rha(1→2)]Glc
 34. 25*R* R₁=H; R₃=Glc; R₂=Rha(1→4)[Rha(1→2)]Glc



35. 25*S* R₁=H; R₃=Glc; R₂=Rha(1→2)Glc
 36. 25*R* R₁=H; R₃=Glc; R₂=Rha(1→2)Glc



30. 25*R* or 25*S* R₁=H; R₃=Glc; R₂=Rha(1→2)Glc

图 5 葫芦巴中皂苷类成分的结构式

Fig. 5 Chemical structures of saponins in fenugreek

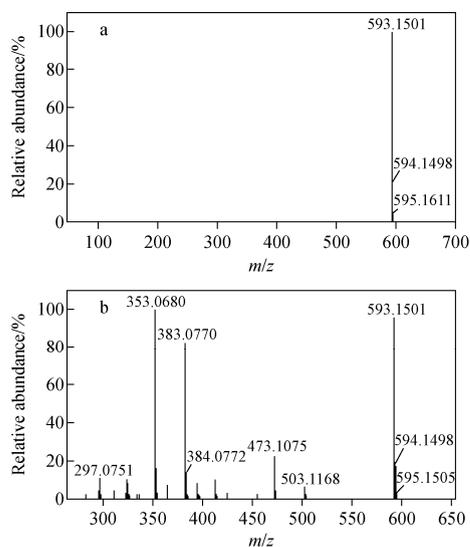


图 6 化合物 13 的 MS(a)和 MS/MS(b)质谱图

Fig. 6 MS (a) and MS/MS (b) spectra of compound 13

3 结论

本研究采用超高效液相色谱-四极杆飞行时间质谱法对葫芦巴醇提物和水提物中的黄酮和皂苷类化合物进行定性分析,并以甘草苷为内标,对黄酮和皂苷类化合物进行半定量分析。定性分析共鉴定出 36 种化合物,包括 12 种黄酮类化合物,24 种皂苷类化合物。以代表性的黄酮类和皂苷类化合物为例,对其结构进行了详细阐述。基于目前标准品种的限制,其他黄酮和皂苷类化合物的结构有待进一步研究。葫芦巴经水提取,除得到多糖外,还可以得到黄酮和皂苷类化合物,但水提物中黄酮和皂苷的含量低于醇提物。该结果可为葫芦巴降血糖药物的开发和药效物质基础研究、以及质量控制标准的制订提供理论依据。

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