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椒目及椒目仁油中 α- 亚麻酸的含量测定研究 *

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摘要 目的:建立同时测定椒目及椒目仁油中 α- 亚麻酸含量的 HPLC 方法。**方法:**用固定相为 Kromasil C18 柱(250 mm× 4.6 mm, 5μm),流动相为乙腈 -1%醋酸溶液(90:10),检测波长为 205nm,流速为 1.0 mL·min^{-1},柱温:25℃,进样量:10 μL,测定椒目及椒目仁油中 α- 亚麻酸的含量;**结果:**α- 亚麻酸在(22～500) μg·mL^{-1} 浓度范围内线性关系良好,5 批椒目和椒目仁油中 α- 亚麻酸的平均含量分别为 4.56%, 32.72%,平均回收率分别为 99.87%,98.97%。**结论:**所建方法操作简便,准确可靠,重现性良好,可有效的控制椒目及椒目仁油的质量。

关键词: 椒目;椒目仁油;HPLC 法;α- 亚麻酸

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Study on the Determination of a-linolenic Acid in the Zanthoxylum Bungeanum Maxim and the Seeds Oil from Zanthoxylum Bungeanum Maxim*

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ABSTRACT Objective: To establish the HPLC method to determine the content of a-linolenic acid in the Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim. **Methods:** With the stationary phase for Kromasil C18 column (250 mm × 4.6 mm, 5 μm), mobile phase of acetonitrile and 1% acetic acid solution (90:10), detection wavelength of 205 nm, flow rate of 1.0 mL min $^{-1}$, column temperature: 25 ℃, sample quantity: 10 uL.to determine the content of a-linolenic acid in the Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim. **Results:** Linear range of a-linolenic acid was (22～500) μg·mL^{-1}, and the average contents of a-linolenic acid were 4.56% and 32.72%, the average recoveries of a-linolenic acid were 99.87% and 98.97% for Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim respectively. **Conclusion:** The method is simple, accurate, reliable and with good reproducibility, which can effectively control the quality of Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim.

Key words: Zanthoxylum bungeanum Maxim; The seeds oil from Zanthoxylum bungeanum Maxim; HPLC; a-linolenic acid

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前言

椒目为芸香科植物花椒(Zantthoxylum bungeanum Maxim·)的成熟种子。椒目又名川椒目,干燥的种子呈类球形,表面黑色有光泽,表皮已脱落者,露出黑色网状纹理,种皮质坚硬。椒目气香,味辛辣[1];椒目仁油为采用压榨法或 CO_2 超临界流体萃取等技术,从椒目的种仁中提取的脂肪油[2]。目前,国内外研究发现,椒目仁油中富含不饱和脂肪酸,其中 α- 亚麻酸对人体健康[3]和疾病治疗[4]方面有重要作用。但《中国药典》[5]及各地方标准尚未收载椒目及椒目仁油,只是有些椒目及椒目仁油制剂相关开发的报道[6,7]。为了进一步对药材及制剂标准的制定提供试验依据和参考,本次试验对椒目及椒目仁油中有效成分的测定方法做了进一步的研究,使其测定结果更加精准,为建立和完善椒目及椒目仁油的质量标准提供参考。

1 材料

1.1 仪器

D200 型电子分析天平(德国 Sartorius 公司); LC-2010AHT 高效液相色谱仪 (日本岛津公司);KQ-300E 型超声波清洗器(昆山市超声仪器有限公司);电热恒温水浴锅(北京科伟永兴仪器有限公司)。

1.2 试药

α- 亚麻酸(批号 111631-200502)购自中国药品生物制品鉴定所;5 批椒目及椒目仁油样品购自久芳(韩城)花椒有限公

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司;超纯水(H_2O):美国 Millipore 纯水器制备;乙腈(色谱纯)购自韩国德山药品工业,其它试剂均为国产分析纯。

2 方法与结果

2.1 色谱条件

色谱柱:Kromasil C18 (250 mm× 4.6 mm, 5 μm);流动相:乙腈 -1%醋酸溶液(90:10);检测波长:205 nm;流速:1.0 mL·min^{-1};柱温:25 ℃;进样量:10 μL。理论塔板数按 α- 亚麻酸峰计算应分别不低于 4000[7]。

2.2 对照品溶液的制备

取 α- 亚麻酸对照品适量,精密称定,加乙醇制成每 1 mL 含 0.2 mg 的 α- 亚麻酸。

2.3 椒目供试品制备

取本品粉末约 1 g,精密称定。置具塞锥形瓶中,先后加入石油醚(60-90 ℃)50 mL,超声提取(功率 300 w,频率 40 kHz)2 次,每次 30 min。过滤,滤至圆底烧瓶内,合并滤液,减压回收石油醚。然后,在圆底烧瓶内加入 0.5 mol/L 的氢氧化钾乙醇溶液 10 mL,回流提取 30 min,放冷,加入酚酞试液 3 滴,加 0.5 mol/L 的盐酸溶液至红色刚好退去,溶液转移至 50 mL 量瓶中,加乙醇洗涤圆底烧瓶,洗涤液并入量瓶中,加乙醇至刻度,摇匀。精密量取 1 mL 置 10 mL 量瓶中,加乙醇至刻度,摇匀,即得[8]。

2.4 椒目仁油供试品制备

取本品约 200 mg,精密称定。置圆底烧瓶内,加入 0.5 mol/L 的氢氧化钾乙醇溶液 10 mL,回流提取 30 min,放冷,加入酚酞试液 3 滴,加 0.5 mol/L 的盐酸溶液至红色刚好退去,溶液转移至 50 mL 量瓶中,加乙醇洗涤圆底烧瓶,洗涤液并入量瓶中,加乙醇至刻度,摇匀。精密量取 1 mL 置 10 mL 量瓶中,加乙醇至刻度,摇匀,即得[8]。

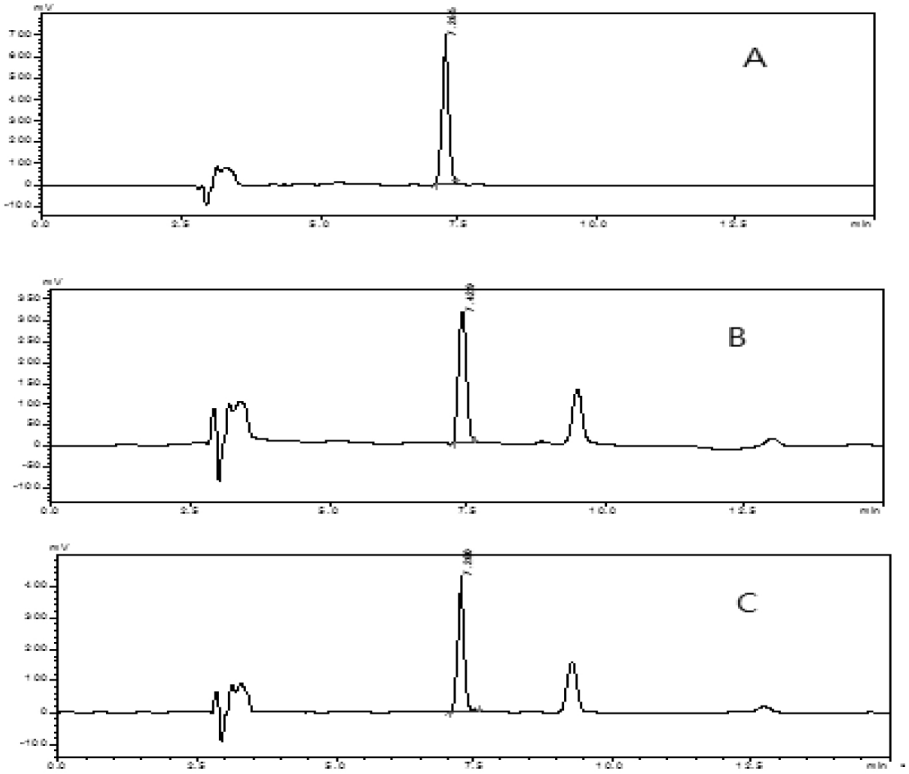

图 1 椒目及椒目仁油样品 HPLC 图

A-α- 亚麻酸对照品 B- 椒目供试品 C- 椒目仁油供试品

Fig.1 HPLC figures of Zanthoxylum bungeanum Maxim and The seeds oil from Zanthoxylum bungeanum Maxim samples

A - a-linolenic acid B-Test samples of Zanthoxylum bungeanum Maxim C - test samples of The seeds oil from Zanthoxylum bungeanum Maxim

2.5 线性关系考察

精密量取 α- 亚麻酸对照品溶液,经乙醇稀释,制成 α- 亚麻酸浓度为 22,110,210,400 和 500 μg·mL^{-1} 的标准品溶液。分别精密吸取 10 μL 注入液相色谱仪按上述色谱条件测定。以浓度(X)为横坐标,峰面积积分值(Y)为纵坐标绘制标准曲线,得 α- 亚麻酸的回归方程为:Y = 33340X-9123.5(r^2 = 0.999 9) 。结果表明,α- 亚麻酸的浓度在(22 ～ 500) μg·mL^{-1} 浓度范围内与各自峰面积积分值呈良好的线性关系。见图 2。

2.6 精密度试验

取同一浓度的标准品溶液,在上述色谱条件下连续进样 6 次,测定峰面积。结果,α- 亚酸峰面积的 RSD 为 0.17%。表明仪器精密度良好。

2.7 重复性试验

取椒目和椒目仁油同一批供试品分别按 2.1.3 和 2.1.4 项下操作方法各制备 5 份样品溶液,进样测定。结果,α- 亚麻酸峰面积的 RSD 值分别为 0.45%、1.64% 。表明本实验所用的方法重复性良好。

2.8 稳定性试验

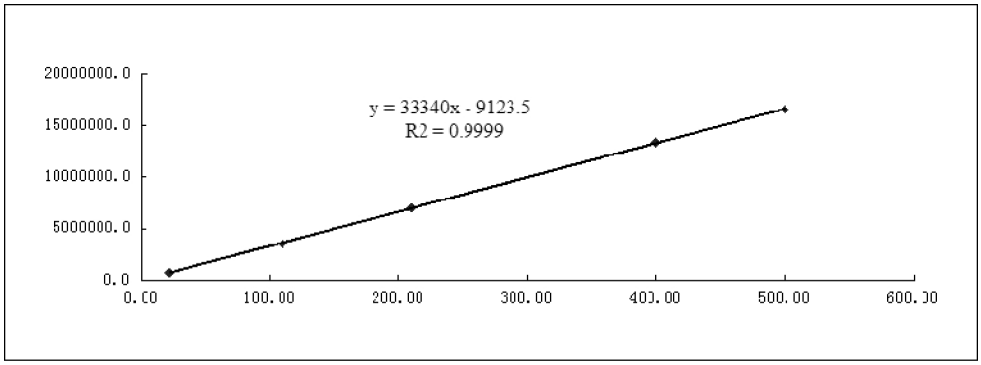

图 2 α- 亚麻酸标准曲线

Fig.2 The standard curve of a-inolenic acid

表 1 椒目加样平均回收率实验结果(n=3)

Table 1 The average recovery experiment results of Zanthoxylum bungeanum Maxim (n = 3)

Technical content (μg)	Add the scalar(μg)	Measured the amount (μg)	Recovery (%)	The average recovery(%)	RSD%
33602.64	20000	53855.81	101.27		
33570.72	20000	53803.19	101.16		
30898.56	20000	50652.69	98.77	99.87	1.24
35454.00	20000	55288.26	99.17		
32923.20	20000	52716.86	98.97		

表 2 椒目仁油加样平均回收率实验结果(n=3)

Table 2 The average recovery experiment results of The seeds oil from Zanthoxylum bungeanum Maxim (n = 3)

Technical content (μg)	Add the scalar(μg)	Measured the amount (μg)	Recovery (%)	The average recovery(%)	RSD%
43779.36	24000	67704.78	99.69		
48589.20	24000	71922.80	97.22		
44531.92	24000	68314.64	99.09	98.97	1.23
46495.12	24000	70153.08	98.57		
46102.48	24000	70172.44	100.29		

取同一浓度对照品溶液,分别于 0、1、2、4、8、12、24 h 测定峰面积,结果,对照品溶液中 α- 亚麻酸峰面积的 RSD 为 0.38%。表明 α- 亚麻酸在 24 h 内稳定。

2.1.9 **加样回收率** 精密称取已知含量的椒目和椒目仁油样品各 5 份,精密添加 α- 亚麻酸对照品,分别按照 2.1.3 和 2.1.4 项下的方法制备供试品溶液,进样测定,计算加样回收率,结果见表 1、2。平均回收率分别为 99.87%、98.97%,RSD 值分别为 1.24%、1.23%。

2.1.10 **样品含量测定** 按上述色谱条件测定 5 批椒目及椒目仁油中 α- 亚麻酸的含量,结果见表 3、4。

3 讨论

表 3 五批椒目样品中 α- 亚麻酸含量测定结果(n=3)

Table 3 Five batches of a-linolenic acid content determination results in the Zanthoxylum bungeanum Maxim samples (n = 3)

Batch number	The average pek areaa	A-linolenic acid content (%)	Average(%)
1	3084326.0	4.59	
2	3111949.0	4.67	
3	3031158.5	4.07	
4	3092718.0	4.61	4.48
5	3090857.5	4.19	
6	3096156.5	4.65	

表 4 五批椒目仁油样品中 α- 亚麻酸含量测定结果(n=3)

Table 4 Five batches of a-linolenic acid content determination results in the seeds oil from Zanthoxylum bungeanum Maxim samples (n=3)

Batch number	The average pek areaa	A-linolenic acid content (%)	Average(%)
1	4481353.5	33.42	31.99
2	4656817.0	33.09	
3	4632539.0	34.55	
4	4410910.0	28.63	
5	4459849.0	30.26	

椒目仁油中富含不饱和脂肪酸,如 α - 亚麻酸、亚油酸等。因其中 α- 亚麻酸含量最高[6,9,10],且作用广泛[11,12],促使对其各方面的研究[13,14]。目前,药材和油样中 α- 亚麻酸的含量测定多为 HPLC 法[9]、气 - 质联用法,也有气相色谱法[15,16],而 HPLC 法测定椒目仁油需经皂化水解、尿素包合所得的脂肪酸甲酯化产物来计算其所含 α- 亚麻酸含量[17],此法比较繁琐,不直观。我们之前用的 HPLC 法测定椒目仁油需经皂化水解, 酸解等步骤, 计算 α- 亚麻酸含量 [7], 后来发现此法不能够准确测定椒目中 α- 亚麻酸的含量。鉴于此,本试验进一步探讨了其制备方法和 HPLC 法的条件,例如,本研究考察了椒目的提取溶剂、提取方法和提取时间, 结果表明用石油醚 (60～90 ℃) 超声提取 30 min 提取效果较好;对供试品溶液的制备考察了提取时间和溶剂用量等,结果表明用氢氧化钾乙醇溶液 10 mL,回流提取 30 min,加酚酞试液 3 滴效果较佳。此外,α- 亚麻酸分子结构中仅含非共轭双键,只有末端吸收,因此选择 205 nm 为检测波长。对于流动相,采用了乙腈 -1%醋酸系统作为流动相,可在保证良好分离度的前提下,避免基线的波动干扰。结果表明:此法不仅能够准确测定其 α- 亚麻酸的含量,且其含量明显提高。

综上,本研究科学、准确地测定了椒目及椒目仁油中 α- 亚麻酸的含量, 为椒目及椒目仁油中提取 α- 亚麻酸提供了新的方法。不仅可以较好地对椒目及椒目仁油的质量进行控制,且为其质量标准的制订提供了参考。

参考文献(References)

[1] 谢克诚. 花椒与椒目不能混淆[J]. 中药材, 1986, (01): 45
Xie Ke-cheng. The peel from Zanthoxylum bungeanum Maximand T-he seeds from Zanthoxylum bungeanum Maxim cannot confuse [J]. Journal of Chinese Medicinal Materials, 1986, (01): 45

[2] 杨倩, 王四旺, 王剑波, 等. 椒目中提取仁油的 3 种方法比较研究[J]. 中国实验方剂学杂志, 2007, 13(3): 20-22
Yang Qian, Wang Si-wang, Wang Jian-bo, et al. Comparative Study of Three M ethods of the Extract Olein from Bunge Pricklyash Seed[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2007, 13(3): 20-22

[3] Gwendolyn Barceló -Coblijn, Eric J. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels[J]. Lipid Researc-h, 2009, 6(48): 355-374

[4] Jeppe Hagstrup Christensen, Erik Berg Schmidt, David Mølenberg, et al. Alpha-linolenic acid and heart rate variability in women examined for coronary artery disease[J]. Original Research Nutrition, Metabolism and Cardiovascular Diseases, 2005, 5(15): 345-351

[5] 国家药典委员会编. 中国药典 2010 年版一部[M]. 中国医药科技出版社, 2010
The State committee of made up pharmacopoeia. The Chinese Pharm-acopeia (2010 edition [1]) [M]. Chinese me dicine science and technology press, 2010

[6] 杨倩, 谢艳华, 贺中民, 等. 高效液相色谱法测定七个不同产地椒目中 α- 亚麻酸的含量[J]. 陕西中医, 2010, 31(11): 1524-1525
Yang Qian, Xie Yan-hua, He Zhong-min, et a1. High-performance liquid chromatography HPLC to seven different origin pepper in the eyes of alpha linolenic acid content [J]. Shaanxi Traditional Chinese Medicine, 2010, 31(11): 1524-1525

[7] 曹蔚, 王四旺, 杨倩, 等. 椒目仁油中 α- 亚麻酸的鉴定和含量测定研究[J]. 科学技术与工程, 2012, 12(3): 22-24
Cao Wei, Wang Si-wang, Yang Qian, et a1. Appraisal and the content determination research of a-linoleic acid from Zanthoxylum bungean-um Maxim seeds oil [J]. Science Technology and Engineering, 2012, 12(3): 22-24

[8] 秦建平, 陆艳芹, 罗雪磊, 等. HPLC 同时测定火麻仁中 α- 亚麻酸、亚油酸和油酸含量[J]. 中国实验方剂学杂志, 2012, 18(7): 71-74
Qin Jian-ping, Lu Yan-qin, Luo Xue-lei, et al. Determination of α-Li-noleic Acid, Linoleic Acid and Oleic Acid in Hemp Seed by HPLC[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2012, 18(7): 71-74

[9] 郝荣荣, 曹蔚, 康荣, 等. 椒目仁油的质量标准研究 [J]. 现代生物医学进展, 2013, 13(3): 541-545
Hao Rong-rong, Cao Wei, Kang Rong, et a1. The Quality Standard of the Seeds Oil from Zanthoxylum Bungeanum Maxim [J]. Progress in Modern Biomedicine, 2013, 13(3): 541-545

[10] 肖会敏, 何悦, 王四旺, 等. 椒目仁油的成分分析及对家兔高血脂模型的影响[J]. 现代生物医学进展, 2011, 11(8): 1454-1457
Xiao Hui-min, He Yue, Wang Si-wang, et a1. Z~ong-minComponent Analysis of semen oll Zanthoxylum bungeanum maxim and Its Efrect on Hyperlipidemic Rabbits' Model[J]. Progress in Modern Biomedic-ine, 2011, 11(8): 1454-1457

[11] 张春娥, 张惠, 刘楚怡, 等. 亚油酸的研究进展 [J]. 粮油加工, 2010, 8(5): 18-21
Zhang Chun-e, Zhang Hui, Liu Chu-yi, et al. Progress of linoleic acid [J]. Cereals and Oils Processing, 2010, 8(5): 18-21

[12] 杨倩, 王四旺, 王剑波, 等. 高纯度 a 一亚麻酸抗血栓活性的初步研究[J]. 现代生物医学进展, 2007, 7(12): 1787-1790
Yang Qian, Wang Si-wang, Wang Jian-bo, et a1. A high purity a lin-olenic acid of antithrombotic active preliminary research[J]. Progress in Modern Biomedicine, 2007, 7(12): 1787-1790

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al standard approach[J]. Circ J, 2011, 75(7): 1656-1660

[9] Spaccarotella C, Mongiardo A, Indolfi C. Pathophysiology of aortic stenosis and approach to treatment with percutaneous valve implantation[J]. Circ J, 2011, 75(1): 11-19

[10] Baumgartner H, Hung J, Bermejo J, et al. Echocardiographicassessment of valve stenosis: EAE/ASE recommendations for clinical practice[J]. J Am Soc Echocardiogr, 2009, 22(1): 1-23; quiz 101-102

[11] Baumgartner H, Otto CM. Aortic stenosis severity: do we need a new concept[J]. J Am Coll Cardiol, 2009, 54(11): 1012-1013

[12] Iung B, Baron G, Butchart EG, et al. A prospective survey of patients with valvular heart disease in Europe: The Euro Heart Survey on Valvular Heart Disease[J]. Eur Heart J, 2003, 24(13): 1231-1243

[13] Carabello BA, Paulus WJ. Aortic stenosis[J]. Lancet, 2009, 373(9667): 956-966

[14] Otto CM, Burwash IG, Legget ME, et al. Prospective study of asymptomatic valvular aortic stenosis. Clinical, echocardiographic, and exercise predictors of outcome[J]. Circulation, 1997, 95(9): 2262-2270

[15] Pellikka PA, Sarano ME, Nishimura RA, et al. Outcome of 622 adults with asymptomatic, hemodynamically significant aortic stenosis during prolonged follow-up[J]. Circulation, 2005, 111(24): 3290-3295

[16] Mihaljevic T, Nowicki ER, Rajeswaran J, et al. Survival after valve replacement for aortic stenosis: implications for decision making[J]. J Thorac Cardiovasc Surg, 2008, 135(6): 1270-1279

[17] Vahanian A, Baumgartner H, Bax J, et al. Guidelines on the management of valvular heart disease. The Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology[J]. Eur Heart J, 2007, 60(6): 1e-50e

[18] Leitman M, Lysyansky P, Sidenko S, et al. Two-dimensional strain-a novel software for real-time quantitative echocardiographic assessment of myocardial function[J]. J Am Soc Echocardiogr, 2004, 17(10):1021-1029

[19] Dandel M, Lehmkuhl H, Knosalla C, et al. Strain and strain rate imaging by echocardiography - basic concepts and clinical applicability[J]. Curr Cardiol Rev, 2009, 5(2): 133-148

[20] Heymans S, Schroen B, Vermeersch P, et al. Increased cardiac expression of tissue inhibitor of metalloproteinase-1 and tissue inhibitor of metalloproteinase-2 is related to cardiac fibrosis and dysfunction in the chronic pressure-overloaded human heart [J]. Circulation, 2005, 112(8): 1136-1144

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[13] Toyoaki Anai, Tomoko Yamada, Takehito Kinoshita, et al. Identification of corresponding genes for three low-α-linolenic acid mutants and elucidation of their contribution to fatty acid biosynthesis in soybean seed[J]. Plant Science, 2005, 6(168): 1615-1623

[14] Moumita Pal M. Ghosh. Studies on comparative efficacy of α-linolenic acid and α-eleostearic acid on prevention of organic mercury-induced oxidative stress in kidney and liver of rat[J]. Food and Chemical Toxicology, 2012, 3-4(59): 1066-107

[15] 王捷频, 李晓晔, 许自超, 等. GC 测椒目仁油中 α- 亚麻酸[J]. 中国新医药, 2003, 2(10): 17-18
Wang Jie-pin, Li Xiao-ye, Xu Zi-chao, et a1. Determination of a-linolenic acid in the seeds oil from Zanthoxylum bungeanum Maxim by GC[J]. China New Medicin, 2003, 2(10): 17-18

[16] 肖会敏, 何悦, 杨倩, 等. GC 法测定椒目仁油中油酸、亚油酸及 α-亚麻酸[J]. 中成药, 2011, 33(8): 1361-1364
Xiao Hui-min, He Yue, Yang Qian, et a1. Determination of Oleic acid, linoleic acid,a-linolenic acid in the Zanthoxylum bungeanum Maxim seeds oil by GC[J]. Chinese Traditional Patent Medicine, 2011, 33(8): 1361-1364

[17] 姚敏娜, 乔雷, 王剑波. 椒目仁油中 α- 亚麻酸的高效液相色谱分析[J]. 陕西中医, 2007, 28(11): 1545-1546
Yao Min-na, Qiao Lei, Wang Jian-bo. Analysis a-linolenic acid in the seeds oil from Zanthoxylum bungeanum Maxim by HPLC[J]. Shaanxi Traditional Chinese Medicine, 2007, 28(11): 1545-1546