大桑菊合剂的提取工艺研究*

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摘要 目的:大桑菊合剂为医院制剂,由桑叶、菊花、薄荷、连翘、鱼腥草等中药经乙醇提取制成,为完善大桑菊合剂的制备工艺,对该制剂的制备工艺进行初步的研究。方法:① 实验设计:以加水量、提取时间、提取次数和醇沉浓度为考察因素,各因素均取3水平,采用正交试验$L_9(3^4)$优选制备工艺。② 含量测定方法:以大桑菊饮合剂中总黄酮的含量为指标,以芦丁为标准品,利用紫外分光光度计于503nm下测定吸光值,计算样品中总黄酮的含量,以此作为筛选大桑菊合剂最佳制备工艺的重要指标。结果:最佳制备工艺为不浸泡,加8倍水,提取3次,每次1小时。结论:经正交设计优化的大桑菊合剂的中药制备工艺合理、可行。

关键词:正交试验;制备工艺;大桑菊合剂
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Study on the Extraction Process of DaSangJu Mixture*

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ABSTRACT Objective: DaSangJu mixture is preparation for the hospital, it is consisted by Folium mori, chrysanthemi flos, Herba menthae, Fructus forsythiae, Bulbus fritillariae thunbergii and other traditional Chinese medicine, and it is extraction by the water, the main object of this study is in order to optimize the extraction of Dasangju mixture. Methods: Experimental Design: The adding amount of water, extracted times, the extraction numbers and ethanol precipitation the concentration of study factors, all factors are taken 3 levels, the use of orthogonal L9 (34) preferred preparation process.Determination: The DaSangJu mixture by the index of total flavonoids, rutin as the standard, by UV spectrophotometer at 503nm absorbance was measured to calculate the total flavonoid content in the sample, as a screening DaSangJu mixture An important indicator of the best preparation. Results: Best Preparation: Do not soak, plus 8 folds of water, extracted 3 times, each for 1 hours. Conclusions: The orthogonal design of DaSangJu mixture of traditional Chinese medicine preparation process is reasonable and feasible.

Key words: Orthogonal design; Preparation; DaSangJu mixture;
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前言

大桑菊合剂为我院的院内制剂,由桑叶、菊花、薄荷、连翘、鱼腥草等中药经乙醇提取制成的合剂,具有疏风解表,清热宣肺,化痰止咳之功效,主要用于治疗风热感冒、咳嗽、肺炎喘嗽等初起见咳嗽、流涕、痰黄、咽痛、发热等症状者。为了进一步完善该制剂的制备工艺,本课题以总黄酮的含量为指标,采用正交实验方法,对该制剂的提取工艺进行了初步研究,现报告如下。

1 仪器与试剂

UV-2501PC 紫外可见分光光度仪(日本岛津),Sartorius 十万分之一电子天平(瑞士),药材(购自广东省药材公司中药饮片厂),芦丁对照品(中国药品生物制品检定所,批号0080-9705),水为超纯水,其余试剂均为分析纯。

2 方法与结果

2.1 提取工艺的条件考察

以加水量、提取时间、提取次数和醇沉浓度为考察因素,各因素均取3水平,以提取液中总黄酮的提取量和得膏率作为评价指标,筛选最佳工艺,因素水平见表1。

2.2 方法

按处方比例,称取药材,均分为9份,按正交试验表L9(34)安排试验好拟定工艺路线进行提取工艺研究。分别收集提取液,回收乙醇,定容,测定总黄酮含量。试验结果见表2、表3。

2.3 标准曲线的绘制

精密称取6mg芦丁标准品至50ml容量瓶,用95%乙醇定容至刻度,即得浓度为0.12mg/ml的芦丁标准对照液。分别吸取2、4、6、8、10ml至25ml容量瓶,分别加蒸馏水稀释至10ml,振荡摇匀后,加入5%亚硝酸钠1ml,摇匀静置6分钟,再加入10%硝酸铝溶液1ml,摇匀静置6分钟,再加4.3%氢氧化钠10ml,摇匀后,加蒸馏水稀释至刻度,摇匀放置15分钟,于503nm下测定吸光值。测定结果及标准曲线见图4。标准曲线

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方程为 Y=0.0789*A-0.00041，相关系数为 0.998。

2.4 样品含量测定

精密吸取提取液 1ml，置 50 ml 容量瓶中，加蒸馏水稀释至刻度，摇匀，精密吸取稀释液 1ml，置 25 ml 容量瓶中，加蒸馏水稀释至 10 ml，振荡摇匀后，加入 5%亚硝酸钠 1ml，摇匀静置 6 分钟，再加入 10%硝酸铝溶液 1ml，摇匀静置 6 分钟，再加 4.3%氢氧化钠 10ml，摇匀后，加蒸馏水稀释至刻度，摇匀放置 15 分钟，于 503nm 下测定吸光值，通过标准曲线回归方程，计算含量。

Tab 1 Factors and levels table

levels	Factors			
	A(adding water) (times)	B(extract time) (hours)	C (extract frequency) (number of times)	D (concentration of alcohol) (%)
1	8	1	1	50%
2	10	1.5	2	60%
3	12	2	3	70%

2.5 最佳工艺验证

按照 $A_1B_1C_3D_3$ 的工艺验证 3 批，测定总黄酮量和干浸膏总量 结果见表 5。由表可知，验证试验结果与正交试验优选结果吻合。

Tab 2 Orthogonal design and results

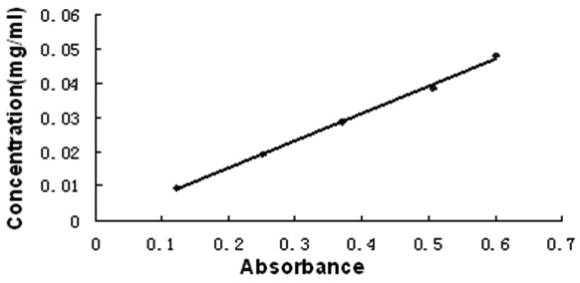
NO	Factors				Indexes of evaluation		Total scores
	A	B	C	D	Total flavonoids X_i (g)	Extracta sicca Y_i (g)	
1	1	1	1	1	0.788	8.05	40
2	1	2	2	2	0.918	9.25	28.923
3	1	3	3	3	1.431	9.40	49.567
4	2	1	2	3	1.171	10.05	28.914
5	2	2	3	1	2.141	10.90	60
6	2	3	1	2	0.958	8.55	40.521
7	3	1	3	2	1.230	9.25	42.759
8	3	2	1	3	0.867	8.85	32.275
9	3	3	2	1	0.922	9.55	24.89
K1	118.4899	111.6731	112.7965	124.8897			
K2	129.4356	121.1981	82.7269	112.2029			
K3	99.9238	114.9780	152.3258	110.7566			
R	9.8373	3.1750	23.1996	4.7110			

综合评分 $=\left(\frac{X_i-X_{min}}{X_{max}-X_{min}}\right)\times 0.6\times 100\%\times\left(\frac{Y_{max}-Y_i}{Y_{max}-Y_{min}}\right)\times 0.4\times 100\%,(i=1,2,3...,9)$

Tab 3 Analysis of variance table

Source of variance	sum of deviation square	degree of freedom	mean square	F value	significance
A	148.3840	2.0000	74.1920	9.5160	＞0.05
B	15.5932	2.0000	7.7966	1.0000	＞0.05
C	812.3069	2.0000	406.1534	52.0938	＜0.05
D	40.3102	2.0000	20.1551	2.5851	＞0.05

注 $F_{0.01(2,2)}$=99.0 $F_{0.05(2,2)}$=19.0



Picture 1 Standard curve of rutin

Tab 4 Verification test

NO	1	2	3	Mean value
Total flavonoids (g)	1.396	1.435	1.427	1.419
Extracta sicca (g)	9.40	9.45	9.30	9.38

3 讨论

3.1 关于考察指标的确定

大桑菊饮合剂由桑叶[1]、菊花[2]、薄荷[3]、桔梗[4]、连翘[5]、鱼腥草[9]、浙贝母[6]、芦根[7]、黄芩[8]、甘草[10]等中药经乙醇提取制成，具有疏风解表，清热宣肺，化痰止咳之功效，主要用于治疗风热感冒、咳嗽、肺炎喘嗽等初起见咳嗽、流涕、痰黄、咽痛、发热等症状者。处方中桑叶、菊花为主要药物，桑叶中含有丰富的异槲皮苷、槲皮苷等活性成分，而黄酮类化合物占干叶重的 1.0%～3.0%，是植物茎叶中黄酮类化合物含量较高的一种[11]。菊花中亦富含黄酮类物质，以 2- 苯基色原酮为基核的化合物，是一类极具有开发前景的天然有机抗氧化剂[12]。目前研究证实，黄酮类成分具有良好的抗菌、抗病毒的作用[13-17]。因此，本研究以合剂的总黄酮含量和干浸膏量为考察指标，采用 $L_9(3^3)$ 正交试验优选提取工艺。

3.2 关于最佳工艺的确定

从表 2 可知，影响提取效果的因素顺序为：提取次数 > 加水量 > 醇沉浓度 > 提取时间，由此得出的最佳工艺组合为 $A_2B_2C_3D_1$。由方差分析结果可知，提取次数对提取结果具有显著性影响，而醇沉浓度、加水量和提取时间对提取结果的影响不大。综合考虑到节约能源，降低生产成本以及成品的澄明度等综合因素，最后确立的工艺为 $A_1B_1C_3D_3$。最终确定最佳工艺为 A1B1C3D3 即：8 倍量水煎煮提取 3 次，每次提取 1 小时，醇沉浓度为 70%。

3.3 本提取工艺易于重复，所得药液真空浓缩至相对密度为 1.10～1.15，合剂符合制剂要求。

4 结论

该提取工艺合理，可行。

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