Bulgarian Chemical Communications, Special Edition H, (pp. 150 – 154) 2017

Analgesic effect of vinegar-processed myrrh and the processing technology research

W.Q. Song[1,2,3,4], X.Q. Wang[1], Y. Qi[1,2,3,4], J. Meng[1,3,4], X.G. Hu[1], S.M. Wang[1,2,3,4], S.W. Liang[1,2,3,4,*]

[1]*College of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, China,*
[2]*Key Laboratory of Digital Quality Evaluation of Chinese Materia Medica, Guangzhou 510006, China*
[3]*Engineering &Technology Research Center for Chinese Materia Medica Quality of the Universities of Guangdong Province, Guangzhou 510006, China*
[4]*Engineering &Technology Research Center for Chinese Materia Medica Quality of Guangdong Province, Guangzhou 510006, China*

Received June 27, 2017; Revised July 20, 2017

The aim of the experiment was to optimize the processing technology of myrrh by orthogonal test, and to make a preliminary pharmacological study on the difference of analgesic effect before and after the processing of myrrh. The method is based on beta-elemene, volatile content and extract oil as the evaluation index, the stir frying temperature, heating time and heating time after the vinegar processed were investigated, the best vinegar processing of myrrh was optimized by testing the comprehensive score of orthogonal optimization, analgesic experiment was performed with the writhing, To investigate whether the analgesic effect were increased after the myrrh processed. Our result is the effect of frying temperature was statistically significant, the best processing of vinegar is preferred to take the net amount of raw myrrh in hot pot, heat gently fry to smoke, 6min, surface micro melting, uniform spraying 5% vinegar, then heated 2min and stir fry until surface light, quickly remove, stand open and cool (every 100kg myrrh, vinegar 5kg), myrrh and vinegar-processed myrrh have analgesic effect, enhance the analgesic effect of vinegar processing, with statistical significance. We can determine the processing parameters of vinegar -processed myrrh, and make it clear that the analgesic effect of vinegar-processed myrrh will be enhanced.

Keywords: myrrh, processing technology, orthogonal test, analgesic effect.

INTRODUCTION

Myrrh is dry resin of Olive plant, it divided into natural myrrh and gum opoponax [5]. Myrrh was found in "medicinal theory". Myrrh is mild, bitter and acrid. Heart liver and spleen meridians, it has good effect for promoting blood circulation to relieve pain, detumescence and promoting granulation. Myrrh mainly contain volatile oil, resin and other ingredients [1]. The study on components of myrrh are mostly concentrated on isolation and identification. Now the main methods of processing of myrrh have stir-baking with vinegar, stir-bake to yellowish and so on. Due to the clinical use of pungent odor, have certain stimulation to the stomach, easy to cause vomiting and nausea. Now the study on the myrrh is more concentrate on volatile oil, including the study on extraction technology and the chemical composition [7]. Myrrh contain volatile oil and resin are effective components[4], and the volatile oil is stimulating, processed with the purpose of eliminating part of volatile oil, reduce the stimulation[3]. But at the same time, reduce the side effects of drugs, whether reduce the effects of analgesic efficacy. How to reduce the loss of myrrh in the course of processing, improve product yield, how to optimize the processing technology of myrrh with efficacy and toxicity experiments, these problems need to be further studied, so as to explore the best processing technology of myrrh, to ensure the safety and effect of clinical application.

MATERIALS AND METHODS

Instrument

Waters 2695 Highly Effective Liquid Chromatography , 2998 PDA; Luna 5 C18 column (4.6 x 250 mm, 5 m); Smart SensorAR550 infrared thermometer (Hongkong SigMa company); KQ-500E ultrasonic cleaner (Kunshan Ultrasonic Instrument Co. Ltd.); Sartorius BP211D 1/10 000 electronic balance, GZX-B40 MBE electric constant temperature drying oven (Shanghai Boxun Industrial Co. Ltd. medical equipment factory); TC-15 constant temperature electric heating jacket (Haining Huaxing instrument factory);RE-52A rotary evaporator (Shanghai Yarong biochemical instrument factory); volatile oil tester.

* To whom all correspondence should be sent:
E-mail: swliang371@126.com

Reagent

Mobile phase: orthophosphoric acid (AR), methanol (chromatographic pure), acetonitrile (chromatographic pure) , water is distilled water; other reagents methanol and ethanol (analytical pure); indometacin Enteric-coated Tablets (batch number: 140101, Guangdong Southern China Pharmaceutical Group Co. Ltd, approval number: Chinese medicine H44020701), acetic acid solution (0.75%); saline, sodium chloride injection (Chinese medicine H13022576, batch number: A14080706); ethanol (medical), glacial acetic acid and other reagents as the pure analysis, β-elemene as reference(National Institute for the control of Pharmaceutical and Biological Products, batch number: 100268-200401). Rice vinegar (purchased from FoShan Haitian seasoning food Limited by Share Ltd, Food production license No: QS 440603023486); Myrrh was purchased from Guangzhou Qingping medicine market, by Professor Li Shuyuan of the Guangdong Pharmaceutical University for the identification of dry resin for the olive branch plant of Commiphora myrrha Engl or Commiphora molmol Engl.

Animals

Kunming mice, both male and female weight 18 ~ 22g, SPF level, provided by experimental animal center of Guangzhou University of Chinese Medicine (Animal license No.: SYXK Guangdong 2014-0081).

Prepare control solution

Precision weighing β-elemene control sample (10.25mg) in 10mL flask. Dissolve and dilute with methanol, shake, as the mother reserved. To learn the 2ml liquor to a 10mL volumetric flask. Dissolve and dilute with methanol to the scale, shake, as control solution.

Prepare test solution

Take 0.2mL volatile oil under the “determination of volatile oil content”. In 10mL flask, dissolved and diluted with methanol to the scale, shake. Over 0.45 m microporous membrane, take proper amount of the filtrate.

METHODS

Orthogonal factor level design

According to preliminary test results, vinegar moxibustion for frying temperature（80℃、100℃、120℃）, heating time（6min, 8min, 10min）and heating time after adding vinegar (2min, 3min, 4min) as main influencing factors of processing. Each factor takes 3 levels, L9 (3^4) orthogonal test was used to design the experiment. The myrrh after election and removal, weighing 9 samples, each of the 200g, according to L9 (3^4) orthogonal experiment method to carry out processing, remove, crush and sift.

Determination of volatile oil content

Take processed product 20g, according to the method of determination of volatile oil to determine volatile oil (Appendix D X B method), results are shown in table.

Measure content of β-elemene

Chromatographic condition

Chromatographic column: Luna 5u C18 column (250 x 4.6 mm, 5 m); the mobile phase: acetonitrile-water (90:10); temperature of column: 30˚C wavelength of detection: 210nm; flow rate: 1mL/min; sample size: 10L.

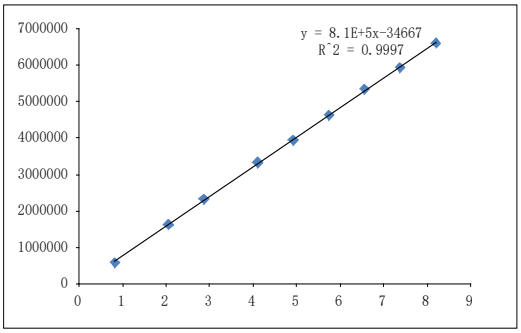

Fig.1. β-elemene standard curve.

Investigate linear relation

Take a amount of β-elemene reference solution under the "Prepare control solution". Samples were 2, 5, 7, 10, 12, 14, 16, 18, 20 L, according to the above conditions to determine, to determine the peak area, the peak area integral value(Y) as the ordinate. With the standard β-elemene injection volume(X) as abscissa. Respectively for linear regression, regression equation: Y=8.1E+5x-34667, R2=0.9997. The results showed that β-elemene in 0.82~8.2g range showed a good linear relationship.

Test the precision

Precision take 10L from β-elemene reference solution. According to the chromatographic conditions (under "chromatographic condition") for sampling 6 times. The calculated RSD value of measured β-elemene peak area was 0.36%.Indicates the instrument has good precision.

Table 1. Results of precision.

Times of sampling	The peak area of β-elemene
1	3366826
2	3347631
3	3349192
4	3348580
5	3373500
6	3371823
RSD (%)	0.36

Test the stability

Take 20g of myrrh powder, precision weighing. Preparation under the "Prepare test solution". In 0, 2, 4, 8, 12, 24 h each injection 10L, with the peak area (A) of β-elemene measured as the index. Calculated relative standard deviation (RSD), To investigate the stability of the sample solution. Obtain β-elemene RSD is 1.81%.The results showed that the sample solution was stable within 24h

Table 2. Results of samples, stability.

Inject time/h	peak area of β-elemene
0	4486596
2	4457576
4	4447117
8	4465825
12	4418967
24	4559992
RSD (%)	1.81

Test the repeatability

Precision weighing the same batch of myrrh powder and divided into 6 parts. Each one is 20g. Preparation under the "chromatographic condition", injection was 10L, with peak area (A) of β-elemene measured as the index, calculated the relative standard deviation (RSD), to investigate the repeatability of the method. To obtain β-elemene RSD = 2.20%.

Table 3. The results of repeatability.

Number	Sample weight/g	peak area A of β-elemene
1	20.0052	2417445
2	20.0015	2441171
3	20.0051	2317036
4	20.0045	24467291
5	20.0042	2364141
6	20.0026	2438017
RSD (%)	---	2.20

Sample recovery

Precision take an amount of control product of β-elemene. Chromatography methanol was added to 0.9533mg/mL as reference solution. Precision weighing known content of myrrh crude 10g, divided into 6 parts. Precisely add the β-elemene control reference solution 3mL. Prepared by the method under the "Prepare test solution ", under "chromatographic condition "measured the chromatographic conditions. The experimental results of β-elemene average recovery rate was 100.07%, RSD was 2.47%.

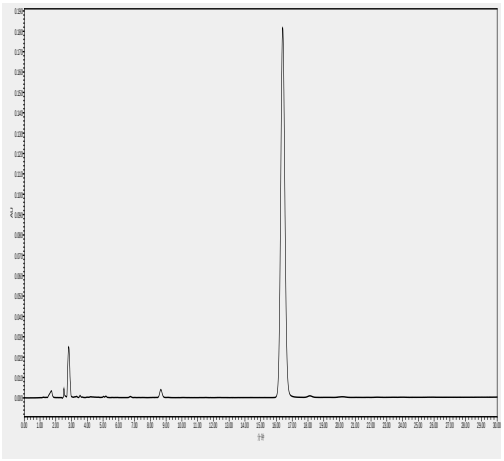

Fig. 2. HPLC diagram of β-elemene of reference substance

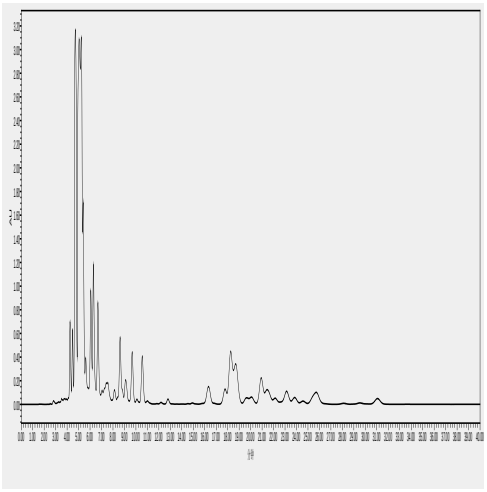

Fig. 3. HPLC diagram of the vinegar-processed myrrh samples.

Measure sample content

Precisely draw control solution and sample solution 10μL. Inject into Liquid chromatograph. Determined by the chromatographic conditions under "chromatographic condition". To calculate the content of β-elemene of each dry sample, the results was shown in Figure 3.

RESEARCH THE SOLVENT OF EXTRACTION

The extract is the index of the dissolution of the pieces, ethanol concentration affects dissolution of active ingredient in vinegar-processed myrrh. Therefore solvent of extraction of the

vinegar-processed myrrh need examined. This study selected 95% ethanol, 75% ethanol, 50% ethanol, 25% ethanol and water as solvent. According to hot-maceration method of 2010 version of Chinese Pharmacopoeia (Appendix X A) [8] to determine. To calculate content of extract after drying.

Table 4. The selection of solvent for extract.

solvent	Content /%
95%ethanol	40.00
75%ethanol	43.13
50%ethanol	37.79
25%ethanol	33.62
water	34.57

As can be seen from table 4, the extracted content of 75% alcohol was the highest. Therefore, using 75% ethanol to extract the extraction of vinegar-processed myrrh.

THE ANALGESIC EFFECTS OF MYRRH AFTER PROCESSING

Prepare samples

The raw products: Take the net amount of myrrh and add 75% ethanol, reflux extracted for 2 times, each time micro boiling for 1hours, cooling for 30min, filtering, merging the filtrate for 2 times, rotary evaporation to recover ethanol. Be condensed liquid medicine which containing crude drug, until the concentration of 65mg/ml, spare.

Vinegar processed products: The processing of vinegar moxibustion has been optimized processing(in the hot pot, heat gently 6min, fry to smoke, surface micro melting, spraying 5% vinegar, then heated 2min, stir fry until surface light). As the concentration of 65mg/ml that liquid medicine containing crude drug and vinegar liquid, spare.

Indomethacin was mixed with normal saline until concentration was 0.325mg/ml.

GROUPING AND ADMINISTRATION

Grouping: 48 mice, free feeding, drink. After 1weeks, meet were randomly and divided into myrrh group, vinegar processed myrrh group, normal saline group, positive control group, 4 groups, and 12 rats in each group, marked with picric acid. Observe and record the writhing times of mice in 15min. Results see table 7.

Drug delivery: fed with myrrh, vinegar processed myrrh and equal volume normal saline, once a day, dose was 20ml/kg. Continuous administration 5days.No drug was given 4 days before the experiment in the indomethacin group. Intraperitoneal injection was given on the fifth day. Each group was treated with alleviating pain test after 1hours of the last administration, that is, intraperitoneal injection of 0.1ml/10g acetic acid (0.75%).

THE INDEX OF ANALGESIC EFFECT OF MYRRH AFTER PROCESSING

By intraperitoneal injection of chemical drugs to mice, the acute peritonitis caused by prolonged pain stimulation, the lethal mice produced writhing response. Because myrrh has the function of promoting blood circulation and relieving pain, can reduce reaction in mice. According to the number of writhing, can judge the analgesic effect of myrrh after processing.

RESULTS

The weighted average method was used to evaluate the processing of vinegar processed myrrh.(the evaluation indicator weighted as follows: β-elemene content evaluation score =the content of β-elemene in each sample x highest content of β-elemene in 40/9 samples; volatile oil content evaluation score= the content of volatile oil in each sample x the highest content of volatile oil in 40/9 samples; alcohol extract content evaluation score = alcohol extract in each sample x the highest content of alcohol extract in 20/9 samples; comprehensive score =β-elemene content evaluation score + alcohol extract content evaluation score —volatile oil content evaluation core). The result is as follows:

Table 5. Results of orthogonal test design

Test number	*A*	*B*	*C*	*D*
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1
K_1	17.050	13.773	12.793	12.703
K_2	11.007	10.653	9.470	11.497
K_3	6.903	10.533	12.697	10.760
R	10.147	3.240	3.323	1.943

* $F_{0.05(2,2)}$=19.000

Table 6. Results of orthogonal design test

Test number	β-elemene /%	Volatile oil content /%	Alcohol extract%	Comprehensive score
1	0.4172	3.64	41.33	21.36
2	0.3303	3.60	41.86	13.71
3	0.3846	3.79	40.17	16.08
4	0.2140	2.84	40.85	10.05
5	0.1937	2.50	41.57	12.10
6	0.2580	3.19	41.41	10.87
7	0.1781	2.55	41.40	9.91
8	0.1599	2.75	41.42	6.15
9	0.2035	3.25	40.62	4.65

* $F_{0.05(2,2)}$=19.000

Table 7. The analgesic effect of processed myrrh

Group	Number of animals	Writhing times	The inhibition ratio
Normal saline group（negative control）	12	24.00±21.51	53.21%
Myrrh processed group	12	11.23±9.21	77.88% *
100℃ vinegar processed myrrh group	12	5.15±9.59	78.53% *
Indomethacin group（positive control）	12	2.23±3.87	90.71% **

Note: t test, compared with physiological saline, * show P<0.05, * * show P<0.01 (If the value of P is less than 0.05,it is significant difference).

From the above analysis of variance showed, the primary and secondary order of the influencing factors of vinegar processed myrrh A (frying temperature) ＞C (heating time after add vinegar) ＞ B (heating time), and the effect of frying temperature was statistically significant. After comprehensive consideration, in accordance with the provisions of the national pharmacopoeia stipulates that vinegar dosage is 5%. Therefore, the optimal processing technology of vinegar processed myrrh was identified as A1B1C1, that is, to take the net amount of raw myrrh, in hot pot, heat gently 6min, fry to smoke, surface micro melting, uniform spraying 5% vinegar, then heated 2min,stir fry until surface light, quickly remove, cool open (each 100kg myrrh with vinegar 5kg).

The analgesic effect of myrrh after processing

According to the results of t test, we can see that the vinegar processed myrrh and myrrh have analgesic effect, analgesic effect of vinegar processed myrrh were enhanced, with statistical significance.

DISCUSSION

At present, research on the processing technology of myrrh is mostly take the content of volatile oil and appearance as the index [6]. But by consulting a large number of documents, it is not clear whether the content of processed myrrh volatile oil significantly reduced and whether it is the effective components [2]. When appearance as the index, due to the lack of objective standard, does not accurately reflect the quality of processed products, but can be used as a reference index. Volatile oil, β-elemene and extracts as 3 indicators for the evaluation of the comprehensive weighted score. By orthogonal test, we can optimum the best processing technology of vinegar processed myrrh. As a reference, the appearance character provides the basis for its quality control. This study belongs to the laboratory experiment, in order to further determine stand – ardized processing of myrrh, .the optimization of the experimental process need the pilot test and to be enlarged.

Myrrh has analgesic effect, but vinegar processed products are better, it has statistical difference between the crude, probably because accessories vinegar also has an analgesic effect, it improve the dissolution rate of other components which have analgesic effect., The reason for the strengthening of vinegar processed myrrh analgesic effect, need to be further studied.

REFERENCE

1. T.B. Ruan, *Journal of Chinese Materia Medica*, **20**(8), 466 (1995).
2. W.H. Li, L. Yang, *Jilin Journal of Traditional Chinese Medicine*, **23**(4), 45 (2003).
3. N.F. Jiao, *Shandong J Tradit Chin Med*, **20**(9), 531 (2001).
4. Y.Q. Sun, G. Wei, L.L. Zhou, *Traditional Chinese medicinal materials*, **24**(8), 566 (2001).
5. R.Q. Hou, *Journal of Shenyang Pharmaceutical University*, **14**(3), 157 (1997).
6. W.H. Chen, *Formulation and technology*, **7**(7), 45 (2010).
7. J.F. Zhao; C.L. Zhou; L. Han. *China Pharmacy*, **22**(7), 661 (2011).
8. Chinese Pharmacopoeia Commission. Pharmacopoeia of People's Republic of China：2010 Edition [M]. Beijing: The Medicine Science and Technology Press of China, Beijing, 2010, app. X A62.