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Original Article

Preparation of total flavonoids from loquat flower and its protective effect on acute alcohol-induced liver injury in mice

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ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form
28 June 2014
Accepted 1 July 2014
Available online 7 November 2014

Keywords:
antioxidant
extraction
hepatoprotection
identification
loquat flower
total flavones

ABSTRACT

This study aimed to research the preparation techniques of total flavones from loquat flower (TFLF), its anti-oxidation capacity, and its protective effect on hepatic injury. The best extraction parameters by orthogonal experimentation were water at 100°C, extraction time 2.5 hours, solid/liquid ratio 1:20, and three decoctions. The chromogenic reaction to the flavones showed that loquat flowers mainly contained flavone, flavonol, and flavanone compounds combining ortho-phenolic hydroxyl group structure in the 10–30% ethanol fraction. The anti-oxidant capacity of $O_2^-\cdot$ was 26.09% and of $OH^-\cdot$ was 83.01% by salicylic acid and pyrogallol auto-oxidation. Compared with the model group, TFLF lowered the levels of alanine aminotransferase, aspartate aminotransferase, triglyceride, and malondialdehyde and liver index significantly, and upregulated the expression of adipose triglyceride lipase and Heine oxygenase-1 mRNA. The present findings suggest that TFLF has protective effect on acute alcoholinduced liver injury in mice and may be related to its antioxidant and free-radical scavenging activity.

1. Introduction

Loquat flower is the dry flower of *Eriobotrya japonica* (Thunb.) Lindl, which belongs to Eriobotrya, Rosaceae. In traditional Chinese medicine, loquat flower is tasteless, tepid, into the lung meridian. Loquat flower has been mainly used to treat common cold, cough, rhinorrhea with clear discharge, immune deficiency, phlegm with blood, and so on. In the countryside, it is commonly used for children who cough with lung heat and patients with persistent cough [1]. China is abundant

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http://dx.doi.org/10.1016/j.jfda.2014.07.001

in loquat, and holds about 70% of this resource worldwide. Flavonoids are a large group of compounds that exist in many plants. They are the aromatic secondary plant metabolites, and are considered important due to their physiological and pharmacological roles and their health effects [2]. It also has been identified that flavonoids are the main chemical components in the loquat flower. Most of them are connected with carbohydrate to form glycosides. However, some exist in the free state (such as aglycone). Flavonoids not only play an important role in the growth, blooming, and fruiting of the plants, but also have a strong effects on antioxidant, antiviral, and anti-inflammatory activities [3], improve bone properties [4], and prevent cardiovascular and cerebrovascular diseases [5].

In this study, the flavonoids of the loquat flower were isolated, and their protective effect on acute alcohol-induced liver injury in mice was examined. The study provides the scientific basis for the research and exploitation of loquat flower resources.

2. Materials and methods

2.1. Plant material

Loquat flowers, which were provided by Shanghai Lian-Yi Loquat Ecological Park, were authenticated to be the dry alabastrum of white loquat belonging to Eriobotrya of Rosaceae by Professor *Hui-ming Wang*, Department of Pharmacognosy, Zhejiang Chinese Medical University, China.

2.2. Animals

Animals used in this study were male Kun Ming mice, weighing 19 ± 1 g provided by the Department of Laboratory Animal Science, Medical Center of Fudan University, Shanghai, China. Animals were fed in a standard animal house at an ambient temperature of 22 ± 2°C and 40–60% relative humidity. Animals were allowed free access to food and water.

2.3. Chemicals

DM301 macroporous adsorptive resin was purchased from Cangzhou Baoen Adsorbing Material Technology (10 Beihai road, Cangzhou, China). Bifendate Pills was purchased from Zhejiang medicine corporation (268 Dengyun road, Hangzhou, China). Triglyceride (TG), alanine aminotransferase (ALT), aspartate amino transferase (AST), and malondialdehyde (MDA) kits were purchased from Nanjing Jiancheng Bioengineering Institute (258-27 Central road, Nanjing, China). All other chemicals used were of analytical grade.

2.4. Determination of the content of total flavonoids

The content of total flavonoids in loquat flowers (TFLF) was detected using the $Al(NO_3)_3$–$NaNO_2$–NaOH colorimetric method [6]. Briefly, rutin was used as standard substance, and was dissolved in methanol to get a concentration of 1 mg/mL. The solution was diluted with distilled water to get the 0.05 mg/mL rutin standard solution. Aliquots of 0.0 mL, 0.4 mL, 0.8 mL, 1.6 mL, 2.4 mL, 3.2 mL, and 4.0 mL of the rutin standard solution were diluted with distilled water to 4.0 mL, and 0.2 mL of 5% $NaNO_2$ was added. After 6 minutes, 0.2 mL of 10% $Al(NO_3)_3$ was added, and then 1.6 mL of 4% NaOH was added and mixed. The absorbance value was measured at 510 nm after 15 minutes. The standard curve was drawn with the abscissa of rutin concentration (0–0.05 mg/mL), and the ordinate of absorbance value. The regression equation was $A = 0.0068C + 0.0017$, $R^2 = 0.9999$. The concentration of rutin (C) and absorbance (A) were in good linear relationship within 0.00–0.05 mg/mL (Fig. 1). Flavonoids content was determined according to the above method.

2.5. Extraction of TFLF

TFLF was extracted according to the procedure of traditional boiling optimized by orthogonal experiment, and macroporous resin chromatography.

The optimal conditions of solid–liquid ratio, extraction temperature, and decocting time were investigated through single factor tests. According to the results of single factor experiments, the identified optimum extracting procedure was optimized with $L_9(3^4)$ orthogonal experimentation. A 60 g sample of crude total flavonoids extracted from loquat flowers using optimal methods was dissolved with distilled water, and loaded on to the DM301 macroporous adsorption resin column (6 cm × 50 cm) at the flow rate of 15 mL/min. Ethanol at 0% (water), 10%, 30%, are 50% was used to elute the column at 25 mL/min. Each fraction of 200 mL was collected and the total volume of stepwise elution was 4000 mL; the concentration of total flavonoids in each fraction was detected, then the concentration of fraction above 80% was collected, and named as TFLF.

2.6. Components analysis of TFLF

2.6.1. HCl–Zn reaction

A 1-mL aliquot of 10% sample ethanol solution and 30 mg Zn dust were added into a test tube and mixed; 100 μL of 10M HCl was added and the color change of the solution observed for several minutes. Most flavone, flavonol, flavanone, and flavanonol compounds will become red to prunosus, and some

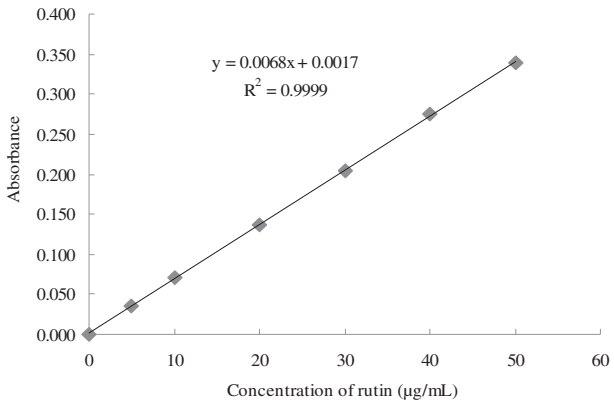

Fig. 1 – Standard curve for total flavonoids detection.