Pharmacognostic and Preliminary Phytochemical Investigations on *Jatrophae curcatis semen*

Cong Li¹, Yuhai Dai², Dingrong Wan¹, Jing Hu¹, Jing Wang¹, *lijun Tang²,³*¹

¹College of Pharmacy, South-Central University for Nationalities, Wuhan, China. ²Hubei Provincial Center for Diseases Control and Prevention, Wuhan, China. ³School of Public Health, Wuhan University, Wuhan, China.

**ABSTRACT**

Introduction: *Jatropha curcas* L. (Euphorbiaceae) distributes in many parts of China with abundant resources. The seed (*Jatrophae curcatis semen*) of this plant is used to produce biodiesel, and the extract also has strong mollusecidal effect on Oncomelania. But few researches have been done about the pharmacognostic and phytochemical nature of the seed.

Methods: The macroscopic and microscopic characters were observed by conventional methods and optical microscope. Moisture content, the alcohol soluble extractive and the fatty oil content were determined respectively. The identification and ingredient analysis were conducted by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Results: The main microscopic characteristics are palisade sclereids, pillar stone cells and filiform sclereids. Moisture content should be not more than 8.0%, alcohol soluble extractive value not less than 14.0%, and content of fatty oil not less than 35.0% according to sample analysis. TLC fingerprinting of the seed extracts was developed.

Conclusion: The results presented can provide some references for authenticity and quality control of *Jatrophae curcatis semen*.

Key word: *Jathrophae curcatis semen*, Pharmacognosy, Phytochemistry

**INTRODUCTION**

*Jatrophae curcatis semen* is the dried mature seed of *Jatropha curcas* L., which is subordinate to Euphorbiaceae. The plant is widely distributed in Yunnan, Sichuan, Guizhou, Guangdong, Guangxi province of China, and the plant is widely cultivated. The main components of the seed are fats, proteins, polypeptides, terpenes and so on [1]. The fats have been extensively researched and utilized as lubricants and biodiesel [2]. The methanol extract of *J. curcatis semen* has molluscicidal effect towards vector freshwater snails, the special host of human schistosomes [3,4]. Hubei Provincial Center for Diseases Control and Prevention also reported the extract of indigenous seed possessed strong inhibition effect on *Oncomelania hupensis* by immersion method. To prevent schistosomiasis in water task, the seed has been developed into external preparations in Hubei province, and the results proved well.

However, a scientific quality specification has not yet been established for the quality control of this crude drug. In view of rich resources and the special medicinal value, studies on the anatomical structure and preliminary physicochemical characteristics of *Jatrophae curcatis semen* were carried out, so as to provide some references of identification and quality evaluation for exploitation and utilization.

**MATERIALS AND METHODS**

Plant materials and reagents

The seeds of *J. curcas* L. were collected from Guangxi Autonomous Region of China and authenticated by one of the authors, Prof. Dingrong Wan, a qualified plant taxonomist.

All reagents used for testing were analytical reagents except chromatographically pure acetonitrile and purified water.

**Morphological studies**

Morphological studies of the crude drugs were performed at macroscopic and microscopic levels. For macroscopic ones, dried mature seeds from different geographical locations were employed. Characters of the material with
respect to shape, size, color, surface, fracture, odour and taste were researched. For microscopic ones, free-hand sections of the seeds were obtained and rendered slightly transparent. The anatomical features such as the structure of testa were observed on transverse sections under the optical microscope. Using light microscopy at appropriate magnification, the dried powder of the seeds were also observed for the diagnostic microscopical features.

**Thin layer chromatographic studies**

2g dried materials were sonicated with 20ml of petroleum ether (90-120°C) for 1h and filtered to remove fat-soluble impurities. Then, the residues of the samples were extracted with 20ml of ethyl acetate by sonicating for 40min and followed with filtration. The filtrate was evaporated to dryness and re-dissolved in 1ml of dehydrated alcohol pending analysis. The extracting solution of reference crude drug of *Jatrophae curcatis semen* was made by employing the method as listed above.6μl of the sample solution and reference crude drug solution, were separately loaded on a silica gel G precoated TLC plate and developed in a mixture of cyclohexane-benzene-ethyl acetate-glacial acetic acid(20:4:6:0.5) by one dimensional ascending method. After development, the plate was air-dried and sprayed with a solution of 10% sulfuric acid in ethanol, then heated at 105°C until the visualization was performed.

**Quantitative determination**

Moisture contents of 10 samples were determined according to the specification of Chinese Pharmacopoeia (2010 Edition, Appendix □ H). Alcohol soluble extractive value of 10 samples were obtained in 95% alcohol using cold maceration method as prescribed by Chinese Pharmacopoeia (2010 Edition, Appendix □ A).

In order to assay content of the fatty oil, 1g of coarse powders of 10 samples were separately extracted with moderate diethyl ether in soxhlet extractor for 8h. Each extract was dried in the evaporating dish with constant weight. Then, the extracts were placed in an oven at 100°C for 1h and moved into a desiccator to cool down for 30min. Finally, the extracts (fatty oil) obtained were accurately weighed and their percentages were calculated in the light of initial air-dried materials.

**RESULTS**

**Macroscopic characteristics**

The seed is elliptical or ovate with a length of 1.6-2.1cm and a width of 0.8-1.2cm. The surface is greyish-black, black or deep blackish-brown in color, and relatively rough, usually possessing many concave spots or fissures in a groove-like shape. One side presents slightly flat, mostly cracked lengthwise into a distinct deep-fissure and many fissures in a groove-like shape, whereas presents arched on the other side. One end of the seed bears a greyish-white, rhomboid hilum and a faintly protruded brownish-black caruncle or a scar of fallen caruncle. The seed has a thick, hard but fragile testa. After stripping the seed coat, a layer of extremely thin, milk-white endopleura connected with endosperm, is visible. The endosperm is thickened, white and oily. The 2 cotyledons are strongly thin. The seed is odorless, possessing a slightly astrinquent taste and can numb the tongue durably.

**Microscopic characteristics**

**Transverse section:** The testa comprises a palisade sclereid layer, parenchyma, a pillar stone cell layer and a filiform sclereid layer. The palisade sclereid layer is a layer of palisade rectangular stone cells, arranged orderly and densely, with relatively thin lateral walls and distinctly thickened outer walls, containing yellowish-brown to blackish-brown substances. The clefts are often found in this sclereid layer, filled with parenchyma cells at the inner side. The parenchymatous tissue consists of several to 10 or more layers of parenchyma cells, with 3-4 layers of relatively small cells at the inner side, often containing clusters of calcium oxalate and laticifers. The pillar stone cell layer has a layer of small, short cylindrical or cylindrical stone cells, arranged regularly and closely, presenting yellowish-brown contents. The filiform sclereid layer is constituted by a layer of slender, fibrous stone cells, frequently curved, arranged radially and densely, containing brownish-black substances. The thin endopleura, separated from the testa, is made up of several layers of flat parenchyma cells, accompanied by vascular bundles at the inner side. The dilapidated tissue exists in the inside of endopleura, is relatively thin, and sometimes separated from endopleura. The cells of endosperm contain oil droplets and aleurone grains. The cotyledons are thin, often detached from endosperm. The parenchyma of endopleura, endosperm and cotyledons all contain a number of fine clusters of calcium oxalate. (Fig.1, 2, 3)

**Powder:** The powder is greyish-yellow to greyish-black in color. There are three types of sclereid. The first one is filiform sclereid, mostly in bundles, densely arranged in order, with even outer edge of outer walls. And the stone cell is rectangular, palisade-like or irregular rectangular, about 60-200μm long and 13-25μm in diameter. The walls are relatively thin on the three sides and outer wall is extremely thickened up to 20μm, in which pits and pit canals are visible, containing yellowish-brown to blackish-brown substances in lumen. The second one is the small pillar stone cell, arranged in a row, and the stone cell is
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irregular short cylindrical, cylindrical, suboblong or irregular oblate in shape, with a length of 8-65μm and a diameter of 8-25μm, obtuse, obtuse-rounded or relatively flattened at ends, walls of which thickened or relatively thickened, sometimes with slightly sparse, distinct pit canals or slender, oblique pits. The last one is slender, fibrous stone cell, often curved, arranged compactly in bundles, mostly broken, and the intact cell is up to 900μm in length and 10-15μm in diameter, obtuse, round-obtuse or truncated at ends, with walls thickened or relatively thickened, relatively dense pits and pit canals, containing blackish-brown substances in lumen. The laticiferous tubes are mostly branched and colorless or brownish-yellow, 20-50μm in diameter. The parenchyma cells of endopleura, endosperm and cotyledons all contain a number of fine clusters of calcium oxalate, 7-20μm in diameter. Oil droplets and aleurone grains exist in the cells of endosperm and cotyledons. Spiral vessels are slender, 8-18μm in diameter. (Fig.4)
TLC identification

The TLC chromatograms of solutions of samples and reference crude drug present the same color spots at corresponding places. (Fig.5)

Quantitative analysis

Moisture content: The results show that the weight loss on drying of 10 samples are respectively 6.9%, 7.0%, 7.3%, 7.5%, 7.3%, 6.7%, 7.7%, 7.4%, 7.5% and 6.3%. Therefore, the moisture content of Jatrophae curcatis semen should be not more than 8.0%.

Alcohol soluble extractive value: The results of 10 samples are separately 18.4%, 15.2%, 14.2%, 18.0%, 16.7%, 13.7%, 15.4%, 14.4%, 15.3% and 16.9%. According to these results, the alcohol soluble extractive value of Jatrophae curcatis semen should be not less than 14.0%.

Fatty oil content: The results of 10 samples are respectively 39.8%, 40.3%, 42.1%, 37.6%, 40.5%, 41.5%, 36.7%, 39.0%, 45.3% and 42.7%. Hence, the content of fatty oil of Jatrophae curcatis semen should be not less than 35.0%.

DISCUSSION

Regarding the microscopic identification, the main microscopical features helpful to correctly identify Jatrophae curcatis semen are the appearances of palisade sclereids, pillar stone cells, filiform sclereids and their shapes in both the transverse of section and the powder.

As for the chemical constituents of Jatrophae curcatis semen, several literatures have reported that the seed contains ursolic acid[8]. But using the TLC identification method, when spotting the solution of ursolic acid used as reference substance to develop with sample solutions, there were no same color spots in chromatogram of samples and reference solution of ursolic acid at the corresponding places. The results were same after repeating the experiments. Consequently, only the solution of reference crude drug was used as reference in the TLC identification. In order to further determine ursolic acid, HPLC method was applied. The powder was made into sample solutions, using ursolic acid as reference substance with an isocratic mobile phase of methyl cyanides: 0.1% phosphoric acid (80:20) to elute. The results showed that there was no chromatographic peak at the same retention time with reference standard (Fig.6). After improving the preparative method of sample solution and chromatographic condition repeatedly, the results were still the same, illustrating that the content of ursolic acid is extremely low, hardly to detect by HPLC.

The seed possesses anti-fungal and insecticide activities, and also used as purgatives. But it has strong toxicity. When people eat 1-2 seeds, they may manifest dizziness, diarrhea, vomiting and epigastrium burning, and more than two are suggested to be life-threatened[9, 10]. Therefore, the seeds are forbidden to take orally, only making the extracts into...
proper dosage forms to poison *Oncomelania*, *Biomphalaria labrinus* and larva, miracidium, cercaria in the infective stage of *Schistosoma*.

**CONCLUSIONS**

The microscopic characters, the parameters of quantitative analysis and thin layer chromatographic studies can be used for the identification and quality control of the crude drug. This crude drug and their adulterants can be authenticated by using these results. However, these findings are part of a comprehensive study of quality control of *Jatrophae curcatis semen*. The parameters of chemical constituents should be further studied to control the quality.

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